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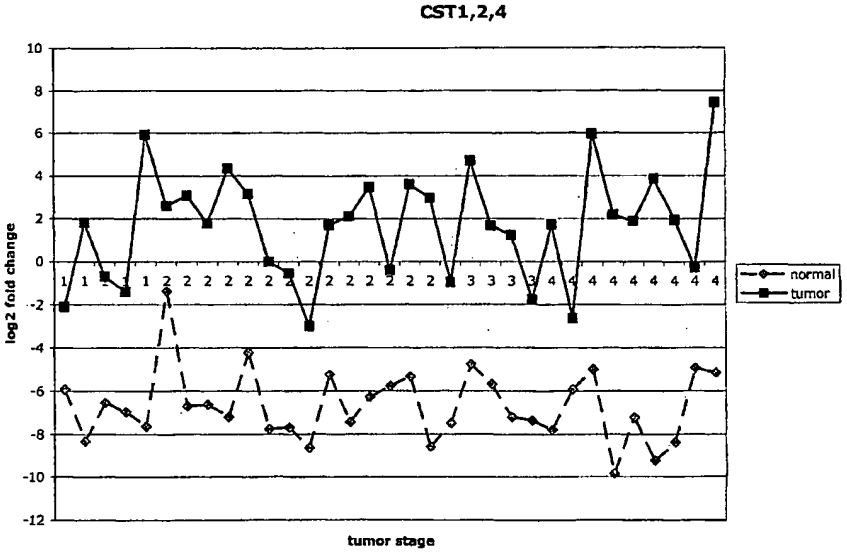

(57) Abstract: Early detection of tumors is a major determinant of survival of patients suffering from tumors, including gastric tumors. Members of the GTM gene family can be over-expressed in gastric tumor tissue and other tumor tissue, and thus can be used as markers for gastric and other types of cancer. GTM proteins can be released from cancer cells, and can reach sufficiently high concentrations in the serum and/or other fluids to permit their detection. Thus, methods and test kits for detection and quantification of GTM can provide a valuable tool for diagnosis of gastric cancer.

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MARKERS FOR DETECTION OF GASTRIC CANCER

Related Application

This application claims priority under 35 U.S.C. 119 to United States Provisional Patent Application Serial No: 60/487,906, filed July 17, 2003, titled "Markers for Detection of Gastric Cancer," listing Parry John Guilford as inventor. The above application is herein incorporated fully by reference.

Field of the Invention

This invention relates to detection of cancer. Specifically, this invention relates to the use of genetic and/or protein markers for detection of cancer, and more particularly to the use of genetic and/or protein markers for detection of gastric cancer.

BACKGROUND

Survival of cancer patients is greatly enhanced when the cancer is detected and treated early. In the case of gastric cancer, patients diagnosed with early stage disease have 5-year survival rates of 90%, compared to approximately 10% for patients diagnosed with advanced disease. However, the vast majority of gastric cancer patients currently present with advanced disease. Therefore, developments that lead to early diagnosis of gastric cancer can lead to an improved prognosis for the patients.

Identification of specific cancer-associated markers in biological samples, including body fluids, for example, blood, urine, peritoneal washes and stool extracts can provide a valuable approach for the early diagnosis of cancer, leading to early treatment and improved prognosis. Specific cancer markers also can provide a means for monitoring disease progression, enabling the efficacy of surgical, radiotherapeutic and chemotherapeutic treatments to be tracked. However, for a number of major cancers, the available markers suffer from insufficient sensitivity and specificity. For example, the most frequently used markers for gastric cancer, ca19-9, ca72-4 and chorioembryonic antigen (CEA) detect only about 15-50% of gastric tumors of any stage, declining to approximately 2-11% for early stage disease. Thus, there is a very high frequency of false negative tests that can lead patients and health care practitioners to believe that no disease exists, whereas in fact, the patient may have

severe cancer that needs immediate attention. Moreover, these markers can give false positive signals in up to 1/3 of individuals affected by benign gastric disease.

SUMMARY OF THE INVENTION

Thus, there is an acute need for better methods for detecting the presence of cancer. Aspects of this invention provide methods, compositions and devices that can provide for detection of early stage cancer, and decreasing the frequency of false positives and false negative test results.

In certain embodiments, molecular analysis can be used to identify genes that are over-expressed in gastric tumor tissue compared to non-malignant gastric tissue. Such analyses include microarray and quantitative polymerase chain reaction (qPCR) methods. Cancer genes and proteins encoded by those genes are herein termed gastric tumor markers (GTM). It is to be understood that the term GTM does not require that the marker be specific only for gastric tumors. Rather, expression of GTM can be increased in other types of tumors, including malignant or non-malignant tumors, including gastric, bladder, colorectal, pancreatic, ovarian, skin (e.g., melanomas), liver, esophageal, endometrial and brain cancers, among others. It should be understood, however that the term GTM does not include prior the art markers, ca19-9, ca72-4 and CEA. Some GTM are sufficiently over-expressed to be diagnostic of gastric cancer with a high degree of reliability, and in other cases, over-expression of two or more GTM can provide reliable diagnosis of gastric cancer.

In certain embodiments, microarray methods can be used to detect patterns of over-expression of one or more genes associated with cancer.

In other embodiments, quantitative polymerase chain reaction (qPCR) can be used to identify the presence of markers over expressed in tumor or other biological samples.

Some of the embodiments of GTM detection disclosed herein are over expressed in a highly selective fashion in tumor cells and little, if at all, in non-tumor cells, permitting sensitive and accurate detection of cancer with measurement of only one over expressed GTM. In other embodiments, over-expression of two, three or more GTM can be detected in a sample and can provide greater certainty of diagnosis.

Selected genes that encode proteins can be secreted by or cleaved from the cell. These proteins, either alone or in combination with each other, have utility as serum or body fluid markers for the diagnosis of gastric cancer or as markers for

monitoring the progression of established disease. Detection of protein markers can be carried out using methods known in the art, and include the use of monoclonal antibodies, polyclonal antisera and the like.

BRIEF DESCRIPTION OF THE FIGURES

This invention is described with reference to specific embodiments thereof and with reference to the figures, in which:

Figure 1 depicts a table of markers and oligonucleotide sequences of markers for gastric cancer of this invention.

Figure 2 depicts a table of results obtained of studies carried out using microarray methods.

Figure 3 depicts a table of results obtained of studies carried out using quantitative PCR.

Figures 4a – 4d depict relationships between log2 fold results obtained using array and qPCR methods, in which the data is centered on the median normal for four gastric cancer markers. Grey squares correspond to non-malignant ("normal") samples and black triangles to tumor samples. Figure 4a: ASPN. Figure 4b: SPP1. Figure 4c: SPARC. Figure 4d: MMP12.

Figures 5a-5w depict histograms showing the relative frequency vs. log2 fold change data obtained from quantitative PCR studies of various tumor markers. Figure 5a: ASPN; Figure 5b: CST1,2 & 4; Figure 5c: CSPG2; Figure 5d: IGFBP7; Figure 5e: INHBA; Figure 5f: LOXL2; Figure 5g: LUM; Figure 5h: SFRP4; Figure 5i: SPARC; Figure 5j: SPP1; Figure 5k: THBS2; Figure 5l: TIMP1; Figure 5m: adlican; Figure 5n: PRS11; Figure 5o: ASAH1; Figure 5p: SFRP2; Figure 5q: GGH; Figure 5r: MMP12; Figure 5s: KLK10; Figure 5t: LEPRE1; Figure 5u: TG; Figure 5v: EFEMP2 and Figure 5w: TGFBI.

Figure 6 is a histogram showing the number of markers with a higher expression than the 95th percentile of the median normal expression. Results are based on qPCR data and are shown separately for each tumor sample.

Figures 7a- 7c depicts graphs that show relative log2 expression of the markers in individual tumor samples and non-malignant samples compared to the expression of the gene for the tumor marker, CEA. CEA is the serum marker currently most used to monitor progression of gastric cancer.

Figure 8 shows a table that complements Figure 3. Figure 8 summarizes expression levels determined by qPCR for the candidate tumor markers, but using the paired data (i.e., tumor ("T") and non-malignant ("N") samples from the same individual) to provide a T:N ratio. Figure 8 also includes additional markers not included in Figure 3, namely MMP2, CGR11, TGFB1, PCSK5, SERPINB5, SERPINH1. For comparison, the expression level of the established serum marker gene, CEACAM5 (CEA), is also shown. 27 of the 29 markers have a median T:N difference greater than or equal to CEA. Further, compared to CEA, 29/29 of the markers have a higher percentage of paired samples in which the expression in the tumor sample exceeds the expression in the normal sample. Three markers, CST1,2,44, ASPN and SFRP4 showed 100% discrimination between the paired tumor and normal samples. The gene sequences of these markers, and the location of the primers and probes used to detect them, are shown herein.

Figures 9a – 9d depict individual and median T:N fold change data for 29 gastric cancer markers in 40 patients with paired samples.

Figures 10a – 10ad depict graphs of tumor stage and log2 fold change in expression of CEA and other GTM of this invention. Figure 10a: adlican; Figure 10b: ASPN; Figure 10c: CSPG2; Figure 10d: CST1,2,4; Figure 10e:EFEMP2; Figure 10f: GGF; Figure 10g: INHBA; Figure 10h: IGFBP7; Figure 10i: KLK10; Figure 10j: LEPRE1; Figure 10k: LUM; Figure 10l: LOXL2; Figure 10m: MMP12; Figure 10n; TIMP1; Figure 10o: ASAH1; Figure 10p: SPP1; Figure 10q: SFRP2; Figure 10r: SFRP4; Figure 10s: SPARC; Figure 10t: PRSS11; Figure 10u: THBS2: Figure 10v: TG; Figure 10w: TGFBI; Figure 10x: CGR11; Figure 10y: SERPINH1; Figure 10z: MMP2; Figure 10aa: PCSK5; Figure 10ab: SERPINB5; Figure 10ac: TGFB1 and Figure 10ad: CEA (CEACAM5).

Figures 11a – 11ad depict graphs of tumor type (diffuse (D) or intestinal (I)) and log2 fold change in expression 29 GTM of this invention and CEA. Figure 11a: adlican; Figure 11b: ASPN; Figure 11c: CSPG2; Figure 11d: CST1,2,4; Figure 11e: EFEMP2; Figure 11f: GGH; Figure 11g: INHBA; Figure 11h: IGFBP7; Figure 11i: KLK10; Figure 11j: LEPRE1: Figure 11k: LUM; Figure 11l: LOXL2; Figure 11m: MMP12; Figure 11n: TIMP1; Figure 11o: ASAH1; Figure 11p: SPP1; Figure 11q: SFRP2; Figure 11r: SFRP4: Figure 11s; SPARC; Figure 11t: PRSS11: Figure 11u: THBS2; Figure 11v: TG; Figure 11w: TGFBI; Figure 11x: CGR11: Figure 11y:

SERPINH1; Figure 11z: MMP2; Figure 11aa: PCSK5; Figure 11ab:SERPINB5; Figure 11ac: TGFB1 and Figure 11ad: CEA (CEACAM5).

Figure 12 depicts a three-dimensional graph showing 3 markers, SERPINH1, CST1,2,4 and INHBA, in a series of gastric tumor samples and non-malignant gastric samples.

Figure 13 depicts a table that shows the effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue. The table has been derived from normal distributions derived from qPCR data.

Figure 14 is a Western blot of 4 tumor markers derived from tumor and non-tumor tissue.

Figure 15 is a Western blot of the tumor marker SPARC in gastric tumor tissue and in serum.

Figure 16 is an immunoblot depicting cystatin SN in the supernatant of a gastric cell line, AGS.

DETAILED DESCRIPTION

Definitions

Before describing embodiments of the invention in detail, it will be useful to provide some definitions of terms as used herein.

The term "GTM" or "gastric tumor marker" or "GTM family member" means a gene, gene fragment, RNA, RNA fragment, protein or protein fragment related or other identifying molecule associated with gastric cancer that does not include molecules that are known in the prior art to be associated with gastric cancer, ca19-9, ca72-4 and CEA. Examples of GTMs are included herein below.

The term "marker" means a molecule that is associated quantitatively or qualitatively with the presence of a biological phenomenon. Examples of "markers" are GTMs, however, "markers" also includes metabolites, byproducts, whether related directly or indirectly to a mechanism underlying a condition.

The term "qPCR" means quantitative polymerase chain reaction.

The term "expression" includes production of mRNA from a gene or portion of a gene, and includes the production of a protein encoded by an RNA or gene or portion of a gene, and includes appearance of a detection material associated with expression. For example, the binding of a binding ligand, such as an antibody, to a gene or other oligonucleotide, a protein or a protein fragment and the visualization of

the binding ligand is included within the scope of the term "expression." Thus, increased density of a spot on an immunoblot, such as a Western blot, is included within the term "expression" of the underlying biological molecule.

The term "CPN2" means human carboxypeptidase N, polypeptide 2, 83 kDa chain; and carboxypeptidase N.

The term "HAPLN4" means human hyaluronan glycoprotein link protein 4.

The term "MMP12" means human matrix metalloproteinase 12.

The term "INHBA" means human inhibin, beta A (also includes activin A, activin AB or alpha polypeptide).

The term "IGFBP7" means human insulin-like growth factor 7.

The term "GGH" means human gamma-glutamyl hydrolase (also known as conjugase, folylpolygammaglutamyl hydrolase).

The term "LEPRE1" means human leucine proline-enriched proteoglycan (also known as leprecan 1).

The term "CST4" means human cystatin S.

The term "SFRP4" means human secreted frizzled-related protein 4.

The term "ASPN" means human asporin (also known as LRR class 1).

The term "CGREF1" or "CGR11" means human cell growth regulator with EF hand domain 1.

The term "KLK" means either human kallikrein 10, variant 1 or human kallikrein 10, variant 2, or both, unless specified otherwise.

The term "TIMP1" means human tissue inhibitor of metalloproteinase 1 (also known as erythroid potentiating activity or collagenase inhibitor).

The term "SPARC" means human secreted protein, acidic, cysteine-rich (also known as osteonectin).

The term "TGFBI" means human transforming growth factor, beta-induced, 68kDa.

The term "EFEMP2" means human EGF-containing fibulin-like extracellular matrix protein 2.

The term "LUM" means human lumican.

The term "SNN" means human stannin.

The term "SPP1" means human secreted phosphoprotein 1 (also known as osteopontin, or bone sialoprotein I, or early T-lymphocyte activation 1).

The term "CSPG2" means human chondroitin sulfate proteoglycan 2 (also known as versican).

The term "ASAH1" means human N-acylsphingosine amidohydrolase, variant 1, or N-acylsphingosine amidohydrolase, variant 2, or both N-acylsphingosine amidohydrolase variants 1 and 2 (also known as acid ceramidase 1, variants 1 and 2).

The term "PRSS11" means human protease, serine, 11 (also known as IGF binding serine protease).

The term "SFRP2" means human secreted frizzled-related protein 2.

The term "PLA2G12B" means human phospholipase A2, group XIIB.

The term "SPON2" means human spondin 2, extracellular matrix protein.

The term "OLFM1" means human olfactomedin 1.

The term "TSRC1" means human thrombospondin repeat containing 1.

The term "THBS2" means human thrombospondin 2.

The term "adlican" means DKFZp564I1922.

The term "CST2" means human cystatin SA.

The term "CST1" means human cystatin SN.

The term "LOXL2" means human lysyl oxidase-like enzyme 2.

The term "TG" means human thyroglobulin.

The term "TGFB1" means human transforming growth factor, beta1 .

The term "SERPINH1" means human serine or cysteine proteinase inhibitor clade H (also known as heat shock protein 47, member 1, or collagen binding protein 1).

The term "SERPINB5" means human serine or cysteine proteinase inhibitor, clade B (also known as ovalbumin, member 5).

The term "CEACAM5" or "CEA" means human carcinoembryonic antigen-related cell adhesion molecule 5.

The term "MMP2" means human matrix metalloproteinase 2 (also known as gelatinase A, or 72 kDa gelatinase, or 72 kDa type IV collagenase).

The term "PCSK5" means human proprotein convertase subtilisin/kexin type 5.

It is to be understood that the above terms may refer to protein, DNA sequence and/or RNA sequence. It is also to be understood that the above terms also refer to non-human proteins, DNA and/or RNA having the same sequences as depicted herein.

Description of Embodiments of the Invention

Markers for detection and evaluation of tumors including gastric cancer are provided that have a greater reliability in detecting gastric cancer than prior art markers. By the term "reliability" we include the absence of false positives and/or false negatives. Thus, with higher reliability of a marker, fewer false positives and/or false negatives are associated with diagnoses made using that marker Therefore, in certain embodiments, markers are provided that permit detection of gastric cancer with reliability greater than the reliability of prior art markers of about 50%. In other embodiments, markers are provided that have reliability greater than about 70%; in other embodiments, greater than about 73%, in still other embodiments, greater than about 80%, in yet further embodiments, greater than about 90%, in still others, greater than about 95%, in yet further embodiments greater than about 98%, and in certain embodiments, about 100% reliability.

Thus, we have surprisingly found numerous genes and proteins whose presence is associated with gastric tumors. Detection of gene products (e.g., oligonucleotides such as mRNA) and proteins and peptides translated from such oligonucleotides therefore can be used to diagnose tumors, such as gastric tumors. Array analysis of samples taken from patients with gastric tumors and from non-malignant tissues of the same subjects has led us to the surprising discovery that in many gastric tumors, specific patterns of over-expression of certain genes are associated with the disease.

Cancer markers can also be detected using antibodies raised against cancer markers.

By analyzing the presence and amounts of expression of a plurality of cancer markers can thus increase the sensitivity of diagnosis while decreasing the frequency of false positive and/or false negative results.

General Approaches to Cancer Detection

The following approaches are non-limiting methods that can be used to detect cancer including gastric cancer using GTM family members.

- Microarray approaches using oligonucleotide probes selective for products of GTM genes.

- Real-time quantitative PCR (qPCR) on tumor samples and normal samples using marker specific primers and probes.
- Enzyme-linked immunological assays (ELISA).
- Immunohistochemistry using anti-marker antibodies on gastric tumors and lymph node metastases.
- Immunohistochemistry using anti-marker antibodies on other tumors including but not limited to colorectal, pancreatic, ovarian, melanoma, liver, esophageal, bladder, endometrial, and brain.
- Immunodetection of marker family members in sera from gastric cancer patients taken before and after surgery to remove the tumor.
- Immunodetection of marker family members in sera from healthy individuals and individuals with non-malignant diseases such as gastritis, ulceration, gastric metaplasia and dysplasia.
- Immunodetection of marker family members in patients with other cancers including but not limited to colorectal, pancreatic, ovarian, melanoma, liver, oesophageal, bladder, endometrial, and brain.
- Detection of markers in body fluids, including serum, lymph, peritoneal fluid, cerebrospinal fluid, synovial fluid and the like.
- Immunodetection of marker family members in gastric fluid, peritoneal washes, urine and stool from gastric cancer patients. Using array methods and/or qPCR.
- Analysis of array or qPCR data using computers. Primary data is collected and fold change analysis is performed by comparison of levels of gastric tumor gene expression with expression of the same genes in non-tumor tissue. A threshold for concluding that expression is increased is provided (e.g., 1.5 x increase, 2-fold increase, and in alternative embodiments, 3-fold increase, 4-fold increase or 5-fold increase). It can be appreciated that other thresholds for concluding that increased expression has occurred can be selected without departing from the scope of this invention. Further analysis of tumor gene expression includes matching those genes exhibiting increased expression with expression profiles of known gastric tumors to provide diagnosis of tumors.

In certain aspects, this invention provides methods for detecting cancer, comprising:

(a) providing a biological sample; and

(b) detecting the over expression of a GTM family member in said sample.

In other aspects, the invention includes a step of detecting over expression of GTM mRNA.

In other aspects, the invention includes a step of detecting over expression of a GTM protein.

In yet further aspects, the invention includes a step of detecting over-expression of a GTM peptide.

In still further aspects, the invention includes a device for detecting a GTM, comprising:

a substrate having a GTM capture reagent thereon; and

a detector associated with said substrate, said detector capable of detecting a GTM associated with said capture reagent, wherein the capture reagent includes an oligonucleotide or an antibody.

Additional aspects include kits for detecting cancer, comprising:

a substrate;

a GTM capture reagent, including one or more of a GTM-specific oligonucleotide and a GTM-specific antibody; and

instructions for use.

Yet further aspects of the invention include method for detecting a GTM using qPCR, comprising:

a forward primer specific for said GTM;

a reverse primer specific for said GTM;

PCR reagents;

a reaction vial; and

instructions for use.

Additional aspects of this invention comprise a kit for detecting the presence of a GTM protein or peptide, comprising:

a substrate having a capture agent for said GTM protein or peptide;

an antibody specific for said GTM protein or peptide;

a reagent capable of labeling bound antibody for said GTM protein or peptide; and

instructions for use.

Additional aspects of this invention include a method for manufacturing a monoclonal antibody, comprising the steps of:

In yet further aspects, this invention includes a method for detecting gastric cancer, comprising the steps of:

providing a sample from a patient suspected of having gastric cancer;

measuring the presence of a GTM protein using an ELISA method.

As described herein, detection of tumors can be accomplished by measuring expression of one or more tumor-specific markers. We have unexpectedly found that the association between increased expression of GTMs and the presence of diagnosed gastric cancer is extremely high. The least significant association detected had a p value of about 1.6×10^{-6}. Many of the associations were significant at p values of less than 10^{-20}. With such a high significance, it may not be necessary to detect increased expression in more than one GTM. However, the redundancy in the GTMs of this invention can permit detection of gastric cancers with an increased reliability.

The methods provided herein also include assays of high sensitivity. qPCR is extremely sensitive, and can be used to detect gene products in very low copy number (e.g., 1 – 100) in a sample. With such sensitivity, very early detection of events that are associated with gastric cancer is made possible.

Methods

The following general methods were used to evaluate the suitability of various approaches to molecular identification of markers associated with gastric tumors.

Tumor Collection

Gastric tumor samples and non-malignant gastric tissues were collected from surgical specimens resected at Seoul National University Hospital, Korea and Dunedin Hospital, New Zealand. Diagnosis of gastric cancer was made on the basis of symptoms, physical findings and histological examination of tissues.

RNA Extraction

In some embodiments, expression of genes associated with gastric tumors was analyzed by determining the changes in RNA from samples taken from tumors. Frozen surgical specimens were embedded in OCT medium. 60μm sections were sliced from the tissue blocks using a microtome, homogenized in a TriReagent: water

(3:1) mix, then chloroform extracted. Total RNA was then purified from the aqueous phase using the RNeasy™ procedure (Qiagen). RNA was also extracted from 16 cancer cell lines and pooled to serve as a reference RNA.

Microarray Slide Preparation

Epoxy coated glass slides were obtained from MWG Biotech AG, Ebersberg, Germany) and were printed with ~30,000 50mer oligonucleotides using a Gene Machines microarraying robot, according to the manufacturer's protocol. Reference numbers (MWG oligo #) for relevant oligonucleotides, and the NCBI mRNA and protein reference sequences are shown in Figure 2. Full DNA sequences of the GTM of this invention are shown herein below.

RNA labeling and Hybridization

cDNA was transcribed from 10μg total RNA using Superscript II reverse transcriptase (Invitrogen) in reactions containing 5-(3-aminoallyl)- 2' deoxyuridine – 5'-triphosphate. The reaction was then de-ionized in a Microcon column before being incubated with Cy3 or Cy5 in bicarbonate buffer for 1 hour at room temperature. Unincorporated dyes were removed using a Qiaquick column (Qiagen) and the sample concentrated to 15ul in a SpeedVac. Cy3 and Cy5 labeled cDNAs were then mixed with Ambion ULTRAhyb buffer, denatured at 100°C for 2 minutes and hybridized to the microarray slides in hybridization chambers at 42°C for 16 hours. The slides were then washed and scanned twice in an Axon 4000A scanner at two power settings to yield primary fluorescence data on gene expression.

Normalization Procedure

To compare expression of cancer genes from tumors and non-cancerous tissues, median fluorescence intensities detected by Genepix™ software were corrected by subtraction of the local background fluorescence intensities. Spots with a background corrected intensity of less than zero were excluded. To facilitate normalization, intensity ratios and overall spot intensities were log-transformed. Log-transformed intensity ratios were corrected for dye and spatial bias using local regression implemented in the LOCFIT™ package. Log-transformed intensity ratios were regressed simultaneously with respect to overall spot intensity and location. The

residuals of the local regression provided the corrected log-fold changes. For quality control, ratios of each normalized microarray were plotted with respect to spot intensity and localization. The plots were subsequently visually inspected for possible remaining artifacts. Additionally, an analysis of variance (ANOVA) model was applied for the detection of pin-tip bias. All results and parameters of the normalization were inserted into a Postgres-database for statistical analysis.

Statistical Analysis

Statistically significant changes in gene expression in tumor samples vs. normal tissues were identified by measured fold changes between arrays. To accomplish this, log2 (ratios) were scaled to have the same overall standard deviation per array. This standardization procedure reduced the average within-tissue class variability. The log2 (ratios) were further shifted to have a median value of zero for each oligonucleotide to facilitate visual inspection of results. A rank-test based on fold changes was then used to improve the noise robustness. This test consisted of two steps: (i) calculation of the rank of fold change (Rfc) within arrays and ii) subtraction of the median (Rfc) for normal tissue from the median(Rfc) for tumor tissue. The difference of both median ranks defines the score of the fold change rank presented in Figure 2. Two additional statistical tests were also performed on this standardized data: 1) Two sample student's t-test, with and without the Bonferroni adjustment and 2) the Wilcoxon test.

Statistical Analysis of Marker Combinations

To determine the value of using combinations of two or three of the markers to discriminate between tumor and non-malignant samples, the qPCR data from 40 paired samples (tumor and non-malignant samples from the same patient) were subjected to the following analysis. Normal distributions for the non-malignant and tumor samples were generated using the sample means and standard deviations. The probability that values taken from the tumor expression data would exceed a defined threshold (e.g., greater than 50%, 70%, 73%, 80%, 90%, 95%, 98%, 99% or 100%) in the non-malignant distribution was then determined (i.e., sensitivity). For combinations of markers, the probability that at least one marker exceeded the threshold was determined.

name	symbol	Applied Biosystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	probe	Seq ID No.
asporin (lrr class 1)	ASPN		AAATACAAAAGGACACATTCAAAGGA	1	TGCTTCTGCAATTCTGATATGGA	23	TTGGAAATGAGTGCAAACCCTCTTGATAATAATG	45
chondroitin sulfate proteoglycan 2 (versican)	CSPG2		GCCAGTGGAATGATGTTTCCC	2	TCTTGGCATTTTCTACAACAGGG	24	AGGAACAGTTGCTTGCAGGCCAGC	46
cystatins SN, SA & S	CST1, 2, 4		AGTCCCAGCCCAACTTGGA	3	GGGAACTTCGTAGATCTGGAAAGA	25	AGCCAGAACTGCAGAAGAAACAGTTGTGC	47
gamma-glutamyl hydrolase	GGH		GTGGCAATGCCGCTGAA	4	TGACAGCAACAACTCAGTAGGAAAA	26	TTCACTGGAGGTCAATTGCACAGCAGAAT	48
insulin-like growth factor binding protein 7	IGFBP7		CAGGTCAGCAAGGGCACC	5	TCACAGCTCAAGTACACCTGGG	27	AGCAAGTGCTCTTCCATAGTGACGCCC	49
kallikrein 10	KLK10		ACAACATGATATGTGCTGGACTGG	6	GAGAGGATGCCTTGGAGGGT	28	CTTGCCAGAGTGACTCTGGAGGCCC	50
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1		CTTGAGTACACGCTGACCTCTTC	7	CCGTGACACAGTTCTGCTTACAG	29	CCATCACAGATCATTACATCCAGGTCCTCA	51
lumican	LUM		GATTCTTGTTCCATAGTGCATCTGC	8	CCAATCAATGCCAGGAAGAGA	30	TAAGGATTCAAACCATTTGCCAAAAATGAGTCTAA G	52
lysyl oxidase-like 2	LOXL2		AGGCCAGCTTCTGCTTGGA	9	CCCTGATCGCCGAGTTG	31	CGTAATTCTTCTGGATGTTCTCTTCACATTCTG	53
matrix metalloproteinase 12	MMP12		GCCTCTCTGCTGATGACATACGT	10	AGTGACAGCATCAAAACTCAAATTG	32	TCAGTCCCTGTATGGAGACCCAAAACAGAA	54
metalloproteinase inhibitor 1	TIMP1		CCAGACCACCTATACCAGCG	11	GGACCTGTGGAAGTATCCGC	33	CAAGATGACCAAGATGTATAAAGGGTTCCAAGC	55
n-acylsphingosine amidohydrolase	ASAH1		CGCAGAACGCCTGCAAA	12	ACAGGACATCATACATGGTTTCAAA	34	TGTCTGAACCGCACCAGCCAAGAGAATA	56
secreted frizzled-related protein 2	SFRP2		CGCTAGCAGCGACCACCT	13	TTTTGCAGGCTTCACATACCTTT	35	CTGCCAGCCACCGAGGAAGCTC	57
secreted protein, acidic, cysteine rich	SPARC		TCTTCCCTGTACACTGGCAGTTC	14	GAAAAAGCGGGTGGTGCA	36	TGGACCAGCACCCCATTGACGG	58
serine protease 11 (IGF binding)	PRSS11		TCGGGAGGCCCCGTTAGTAA	15	AAGGAGATTCCAGCTGTCACTTTC	37	AGTGTTAATTCCAATCACTTCACCGTCCAGG	59
thrombospondin 2	THBS2		TGGAAGGACTACACGGCCTATAG	16	TAGGTTTGGTCATAGATAGGTCCTGAGT	38	AGGCCCAAGACCGGCTACATCAGAGTC	60
thyroglobulin	TG		GACGGTTCCTCGCAGTTCAA	17	TGTAAACCGCTCCACTTCACAT	39	TCTGGCAGATTCCGATGCCCACAA	61
human cell growth regulator with EF hand domain 1	CGR11		CTGCCCACCCCTTCCA	18	TTCTGTCCTTCCTAGTCCCTTAGG	40	CCAGCCCAGGAGCAGCTCGG	62
human serine or cysteine proteinase inhibitor clade B	SERPINB5		TCCACGCATTTTCCAGGATAA	19	AAGCCGAATTTGCTAGTTGCA	41	TGACTCCAGGCCCCGCAATGGA	63
transforming growth factor β1	TGFB1		GGTCCATGTCATCACCAATGTT	20	TCTGCAAGTTCATCCCCTCTTT	42	CAGCCTCCAGCCAACAGACCTCAGG	64
human proprotein convertase subtilisin/kexin type 5	PCSK5		AAAAATCTTTGCCGGAAATGC	21	AGTCCTGGCCGTTGAAATACC	43	ACAGAATGTAGGGATGGGTTAAGCCTGCA	65
matrix metalloproteinase 2	MMP2		TTGATGGCATCGCTCAGATC	22	TGTCACGTGGCGTCACAGT	44	TTCAAGGACCGGTTCATTTGGCG	66
human serine or cysteine proteinase inhibitor clade H adlican	SERPINH1	Hs00241844_m1						
	-	Hs00377849_m1						
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	Hs00213545_m1						
secreted frizzled-related protein 4	SFRP4	Hs00180066_m1						
inhibin beta A chain	INHBA	Hs00170103_m1						
osteopontin	SPP1	Hs00167093_m1						
transforming growth factor B-induced	TGFBI	Hs00165908_m1						

Figure 1

Table 1

Quantitative Real-Time PCR

In other embodiments, real-time, or quantitative PCR (qPCR) can be used for absolute or relative quantitation of PCR template copy number. Taqman™ probe and primer sets were designed using Primer Express V 2.0™ (Applied Biosystems). Where possible, all potential splice variants were included in the resulting amplicon, with amplicon preference given to regions covered by the MWG-Biotech-derived microarray oligonucleotide. Alternatively, if the target gene was represented by an Assay-on-Demand™ expression assay (Applied Biosystems) covering the desired amplicons, these were used. The name of the gene, symbol, the Applied Biosystems "assay on demand" number, forward primer, reverse primer and probe sequence used for qPCR are shown in Table 1 and in Figure 1. In the in-house designed assays, primer concentration was titrated using a SYBR green labeling protocol and cDNA made from the reference RNA. Amplification was carried out on an ABI Prism™ 7000 sequence detection system under standard cycling conditions. When single amplification products were observed in the dissociation curves, standard curves were generated over a 625-fold concentration range using optimal primer concentrations and 5'FAM - 3'TAMRA phosphate Taqman™ probe (Proligo) at a final concentration of 250nM. Assays giving standard curves with regression coefficients over 0.98 were used in subsequent assays. It can be appreciated that in other embodiments, regression coefficients need not be as high. Rather, any standard curve can be used so long as the regression coefficients are sufficiently high to permit statistically significant determination of differences in expression. Such regression coefficients may be above about 0.7, above about 0.8, above about 0.9 or above about 0.95 in alternative embodiments.

Assays were performed over two 96 well plates with each RNA sample represented by a single cDNA. Each plate contained a reference cDNA standard curve, over a 625-fold concentration range, in duplicate. Analysis consisted of calculating the ΔCT (target gene CT – mean reference cDNA CT). ΔCT is directly proportional to the negative log2 fold change. Log2 fold changes relative to the median non-malignant log2 fold change were then calculated (log2 fold change – median normal log2 fold change). These fold changes were then clustered into frequency classes and graphed.

Microarray Analysis of Cancer Marker Genes

RNA from 58 gastric tumors and 58 non-malignant ("normal") gastric tissue samples were labeled with Cy5 and hybridized in duplicate or triplicate with Cy3 labeled reference RNA. After normalization, the change in expression in each of 29,718 genes was then estimated by three measures: (i) fold change: the ratio of the gene's median expression (un-standardized) in the tumor samples divided by the median level in the non-malignant samples. (ii) fold change rank and (iii) the statistical probability that the observed fold changes were significant.

Selection of Serum Markers for Gastric Malignancy

In certain embodiments, the cancer marker can be found in biological fluids, including serum. Serum markers were selected from the array data based on (i) the presence of a signal sequence characteristic of secreted proteins or cleaved from the outside of the membrane, (ii) the median level of over-expression (fold change) in tumors compared to non-malignant controls, (iii) the median change in expression rank between tumors and non-malignant controls, and (iv) the degree of overlap between the ranges of expression in the tumor and the non-malignant controls.

All 29 GTMs are known to have a signal peptide sequence at the 5'end of their coding sequences. The signal sequence targets the GTM proteins for transport to an extracellular compartment through the plasma membrane (Gunner von Heijne, Journal of Molecular Biology 173:243-251 (1984). In addition, none of the GTMs have transmembrane sequence motifs that would result in the full-length protein being retained within the plasma membrane. Consequently, all of the GTM markers of this invention are likely to be secreted into the extracellular compartment, and therefore can be in contact with the vasculature, either being taken up by capillaries, or by being transported into the lymphatic system and then into the vasculature. As a result, each of these tumor-derived markers will be present in the blood.

Next, genes were excluded if >50% of the tumor samples showed expression levels within the 95th percentile of the non-malignant range. The variation in the degree of over-expression in the tumor samples reflects not only tumor heterogeneity but also variations in the extent of contamination of the tumor samples with "normal" tissue including muscle, stromal cells and non-malignant epithelial glands. This "normal" contamination ranged from 5 to 70% with a median of approximately 25%. Other genes were excluded because of high relative expression in hematopoietic cells,

or elevated expression in metaplastic gastric tissue. It can be appreciated that depending on the degree of contamination by normal cells or cells that normally express the marker, different threshold ranges can be selected that can provide sufficient separation between a cancer source and a normal source.

GTM that we have found to be useful include genes (DNA), complementary DNA (cDNA), RNA, proteins, and protein fragments of the following markers: carboxypeptidase N, polypeptide 2, 83 kDa chain (also known as carboxypeptidase N (CPN2), matrix metalloproteinase 12 (MMP12), inhibin ("INHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline-enriched proteoglycan ("LEPRE1"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein (KLK10), tissue inhibitor of metalloproteinase 1 ("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor, β-induced ("TGFBI"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAH1"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlican, cystatin SA ("CST2"), cystatin SN (CST1), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta1 ("TGFB1"), serine or cysteine proteinase inhibitor clade H ("SERPINH1"), serine or cysteine proteinase inhibitor clade B ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5"), and hyalronan proteoglycan link protein 4 ("HAPLN4").

DNA sequences of GTM of this invention along with identifying information are shown herein below.

Matrix Metalloproteinase 12

>gi|4505206|ref|NM_002426.1| Homo sapiens matrix metalloproteinase 12 (macrophage elastase) (MMP12), mRNA | qPCR forward_primer match [758..780] | qPCR reverse_primer match [888..864] | qPCR probe match [786..815]

TAGAAGTTTACAATGAAGTTTCTTCTAATACTGCTCCTGCAGGCCA
CTGCTTCTGGAGCTCTTCCCCTGAACAGCTCTACAAGCCTGGAAAAAAAT
AATGTGCTATTTGGTGAGAGATACTTAGAAAAATTTTATGGCCTTGAGATA
AACAAACTTCCAGTGACAAAAATGAAATATAGTGGAAACTTAATGAAGG
AAAAAATCCAAGAAATGCAGCACTTCTTGGGTCTGAAAGTGACCGGGCAA
CTGGACACATCTACCCTGGAGATGATGCACGCACCTCGATGTGGAGTCCC
CGATCTCCATCATTTCAGGGAAATGCCAGGGGGGCCCGTATGGAGGAAAC
ATTATATCACCTACAGAATCAATAATTACACACCTGACATGAACCGTGAG
GATGTTGACTACGCAATCCGGAAAGCTTTCCAAGTATGGAGTAATGTTAC
CCCCTTGAAATTCAGCAAGATTAACACAGGCATGGCTGACATTTTGGTGG
TTTTTGCCCGTGGAGCTCATGGAGACTTCCATGCTTTTGATGGCAAAGGTG
GAATCCTAGCCCATGCTTTTGGACCTGGATCTGGCATTGGAGGGGATGCA
CATTTCGATGAGGACGAATTCTGGACTACACATTCAGGAGGCACAAACTT
GTTCCTCACTGCTGTTCACGAGATTGGCCATTCCTTAGGTCTTGGCCATTCT
AGTGATCCAAAGGCTGTAATGTTCCCCACCTACAAATATGTCGACATCAA
CACATTTCGCCTCTCTGCTGATGACATACGTGGCATTCAGTCCCTGTATGG
AGACCCAAAAGAGAACCAACGCTTGCCAAATCCTGACAATTCAGAACCAG
CTCTCTGTGACCCCAATTTGAGTTTTGATGCTGTCACTACCGTGGGAAATA
AGATCTTTTTCTTCAAAGACAGGTTCTTCTGGCTGAAGGTTTCTGAGAGAC
CAAAGACCAGTGTTAATTTAATTTCTTCCTTATGGCCAACCTTGCCATCTG
GCATTGAAGCTGCTTATGAAATTGAAGCCAGAAATCAAGTTTTTCTTTTTA
AAGATGACAAATACTGGTTAATTAGCAATTTAAGACCAGAGCCAAATTAT
CCCAAGAGCATACATTCTTTTGGTTTTCCTAACTTTGTGAAAAAAATTGAT
GCAGCTGTTTTTAACCCACGTTTTTATAGGACCTACTTCTTTGTAGATAAC
CAGTATTGGAGGTATGATGAAAGGAGACAGATGATGGACCCTGGTTATCC
CAAACTGATTACCAAGAACTTCCAAGGAATCGGGCCTAAAATTGATGCAG
TCTTCTATTCTAAAAACAAATACTACTATTTCTTCCAAGGATCTAACCAAT
TTGAATATGACTTCCTACTCCAACGTATCACCAAAACACTGAAAAGCAAT
AGCTGGTTTGGTTGTTAGAAATGGTGTAATTAATGGTTTTTGTTAGTTCAC
TTCAGCTTAATAAGTATTTATTGCATATTTGCTATGTCCTCAGTGTACCACT
ACTTAGAGATATGTATCATAAAAATAAAATCTGTAAACCATAGGTAATGA
TTATATAAAATACATAATATTTTTCAATTTTGAAAACTCTAATTGTCCATTC
TTGCTTGACTCTACTATTAAGTTTGAAAATAGTTACCTTCAAAGCAAGATA
ATTCTATTTGAAGCATGCTCTGTAAGTTGCTTCCTAACATCCTTGGACTGA
GAAATTATACTTACTTCTGGCATAACTAAAATTAAGTATATATATTTTGGC
TCAAATAAAATTG SEQ ID NO:67

Inhibin Beta A

>gi|4504698|ref|NM_002192.1| Homo sapiens inhibin, beta A (activin A, activin AB alpha polypeptide) (INHBA), mRNA | qPCR assay_on_demand_context match [457..481]

TCCACACACACAAAAAACCTGCGCGTGAGGGGGGAGGAAAAGCAG
GGCCTTTAAAAAGGCAATCACAACAACTTTTGCTGCCAGGATGCCCTTGCT
TTGGCTGAGAGGATTTCTGTTGGCAAGTTGCTGGATTATAGTGAGGAGTTC
CCCCACCCCAGGATCCGAGGGGCACAGCGCGGCCCCCGACTGTCCGTCCT
GTGCGCTGGCCGCCCTCCCAAAGGATGTACCCAACTCTCAGCCAGAGATG
GTGGAGGCCGTCAAGAAGCACATTTTAAACATGCTGCACTTGAAGAAGAG
ACCCGATGTCACCCAGCCGGTACCCAAGGCGGCGCTTCTGAACGCGATCA

GAAAGCTTCATGTGGGCAAAGTCGGGGAGAACGGGTATGTGGAGATAGA
GGATGACATTGGAAGGAGGGCAGAAATGAATGAACTTATGGAGCAGACC
TCGGAGATCATCACGTTTGCCGAGTCAGGAACAGCCAGGAAGACGCTGCA
CTTCGAGATTTCCAAGGAAGGCAGTGACCTGTCAGTGGTGGAGCGTGCAG
AAGTCTGGCTCTTCCTAAAAGTCCCCAAGGCCAACAGGACCAGGACCAAA
GTCACCATCCGCCTCTTCCAGCAGCAGAAGCACCCGCAGGGCAGCTTGGA
CACAGGGGAAGAGGCCGAGGAAGTGGGCTTAAAGGGGGAGAGGAGTGA
ACTGTTGCTCTCTGAAAAAGTAGTAGACGCTCGGAAGAGCACCTGGCATG
TCTTCCCTGTCTCCAGCAGCATCCAGCGGTTGCTGGACCAGGGCAAGAGC
TCCCTGGACGTTCGGATTGCCTGTGAGCAGTGCCAGGAGAGTGGCGCCAG
CTTGGTTCTCCTGGGCAAGAAGAAGAAGAAAGAAGAGGAGGGGGAAGGG
AAAAAGAAGGGCGGAGGTGAAGGTGGGGCAGGAGCAGATGAGGAAAAG
GAGCAGTCGCACAGACCTTTCCTCATGCTGCAGGCCCGGCAGTCTGAAGA
CCACCCTCATCGCCGGCGTCGGCGGGGCTTGGAGTGTGATGGCAAGGTCA
ACATCTGCTGTAAGAAACAGTTCTTTGTCAGTTTCAAGGACATCGGCTGGA
ATGACTGGATCATTGCTCCCTCTGGCTATCATGCCAACTACTGCGAGGGTG
AGTGCCCGAGCCATATAGCAGGCACGTCCGGGTCCTCACTGTCCTTCCACT
CAACAGTCATCAACCACTACCGCATGCGGGGCCATAGCCCCTTTGCCAAC
CTCAAATCGTGCTGTGTGCCCACCAAGCTGAGACCCATGTCCATGTTGTAC
TATGATGATGGTCAAAACATCATCAAAAAGGACATTCAGAACATGATCGT
GGAGGAGTGTGGGTGCTCATAGAGTTGCCCAGCCCAGGGGGAAAGGGAG
CAAGAGTTGTCCAGAGAAGACAGTGGCAAAATGAAGAAATTTTTAAGGTT
TCTGAGTTAACCAGAAAAATAGAAATTAAAAACAAAACAAAACAAAAAA
AAAAACAAAAAAAAAACAAAAGTAAATTAAAAACAAACCTGATGAAACAG
ATGAAACAGATGAAGGAAGATGTGGAAATCTTAGCCTGCCTTAGCCAGGG
CTCAGAGATGAAGCAGTGAAGAGACAGATTGGGAGGGAAAGGGAGAATG
GTGTACCCTTTATTTCTTCTGAAATCACACTGATGACATCAGTTGTTTAAA
CGGGGTATTGTCCTTTCCCCCCTTGAGGTTCCCTTGTGAGCTTGAATCAAC
CAATCTGATCTGCAGTAGTGTGGACTAGAACAACCCAAATAGCATCTAGA
AAGCCATGAGTTTGAAAGGGCCCATCACAGGCACTTTCCTAGCCTAAT

SEQ ID NO:68

Insulin-Like Growth Factor Binding Protein 7

>gi|4504618|ref|NM_001553.1| Homo sapiens insulin-like growth factor binding protein 7 (IGFBP7), mRNA | qPCR forward_primer match [470..487] | qPCR reverse_primer match [567..546] | qPCR probe match [492..517]

GCCGCTGCCACCGCACCCCGCCATGGAGCGGCCGTCGCTGCGCGCC
CTGCTCCTCGGCGCCGCTGGGCTGCTGCTCCTGCTCCTGCCCCTCTCCTCTT
CCTCCTCTTCGGACACCTGCGGCCCCTGCGAGCCGGCCTCCTGCCCGCCCC
TGCCCCCGCTGGGCTGCCTGCTGGGCGAGACCCGCGACGCGTGCGGCTGC
TGCCCTATGTGCGCCCGCGGCGAGGGCGAGCCGTGCGGGGGTGGCGGCGC
CGGCAGGGGGTACTGCGCGCCGGGCATGGAGTGCGTGAAGAGCCGCAAG
AGGCGGAAGGGTAAAGCCGGGGCAGCAGCCGGCGGTCCGGGTGTAAGCG
GCGTGTGCGTGTGCAAGAGCCGCTACCCGGTGTGCGGCAGCGACGGCACC
ACCTACCCGAGCGGCTGCCAGCTGCGCGCCGCCAGCCAGAGGGCCGAGA
GCCGCGGGGAGAAGGCCATCACCCAGGTCAGCAAGGGCACCTGCGAGCA
AGGTCCTTCCATAGTGACGCCCCCCAAGGACATCTGGAATGTCACTGGTG
CCCAGGTGTACTTGAGCTGTGAGGTCATCGGAATCCCGACACCTGTCCTCA

TCTGGAACAAGGTAAAAAGGGGTCACTATGGAGTTCAAAGGACAGAACT
CCTGCCTGGTGACCGGGACAACCTGGCCATTCAGACCCGGGGTGGCCCAG
AAAAGCATGAAGTAACTGGCTGGGTGCTGGTATCTCCTCTAAGTAAGGAA
GATGCTGGAGAATATGAGTGCCATGCATCCAATTCCCAAGGACAGGCTTC
AGCATCAGCAAAAATTACAGTGGTTGATGCCTTACATGAAATACCAGTGA
AAAAAGGTGAAGGTGCCGAGCTATAAACCTCCAGAATATTATTAGTCTGC
ATGGTTAAAAGTAGTCATGGATAACTACATTACCTGTTCTTGCCTAATAAG
TTTCTTTTAATCCAATCCACTAACACTTTAGTTATATTCACTGGTTTTACAC
AGAGAAATACAAAATAAAGATCACACATCAAGACTATCTACAAAAATTTA
TTATATATTTACAGAAGAAAAGCATGCATATCATTAAACAAATAAAATAC
TTTTTATCACAAAAAAAAAAAAAAAA SEQ ID NO: 69

Gamma-Glutamyl Hydrolase

>gi|4503986|ref|NM_003878.1| Homo sapiens gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase) (GGH), mRNA | qPCR forward_primer match [531..547] | qPCR reverse_primer match [611..587] | qPCR probe match [549..577]

TGCCGCAGCCCCCGCCCGCCCGCAGAGCTTTTGAAAGGCGGCGGG
AGGCGGCGAGCGCCATGGCCAGTCCGGGCTGCCTGCTGTGCGTGCTGGGC
CTGCTACTCTGCGGGGCGGCGAGCCTCGAGCTGTCTAGACCCCACGGCGA
CACCGCCAAGAAGCCCATCATCGGAATATTAATGCAAAAATGCCGTAATA
AAGTCATGAAAAACTATGGAAGATACTATATTGCTGCGTCCTATGTAAAG
TACTTGGAGTCTGCAGGTGCGAGAGTTGTACCAGTAAGGCTGGATCTTAC
AGAGAAAGACTATGAAATACTTTTCAAATCTATTAATGGAATCCTTTTCCC
TGGAGGAAGTGTTGACCTCAGACGCTCAGATTATGCTAAAGTGGCCAAAA
TATTTTATAACTTGTCCATACAGAGTTTTGATGATGGAGACTATTTTCCTGT
GTGGGGCACATGCCTTGGATTTGAAGAGCTTTCACTGCTGATTAGTGGAG
AGTGCTTATTAACTGCCACAGATACTGTTGACGTGGCAATGCCGCTGAACT
TCACTGGAGGTCAATTGCACAGCAGAATGTTCCAGAATTTTCCTACTGAGT
TGTTGCTGTCATTAGCAGTAGAACCTCTGACTGCCAATTTCCATAAGTGGA
GCCTCTCCGTGAAGAATTTTACAATGAATGAAAAGTTAAAGAAGTTTTTC
AATGTCTTAACTACAAATACAGATGGCAAGATTGAGTTTATTTCAACAAT
GGAAGGATATAAGTATCCAGTATATGGTGTCCAGTGGCATCCAGAGAAAG
CACCTTATGAGTGGAAGAATTTGGATGGCATTTCCCATGCACCTAATGCTG
TGAAAACCGCATTTTATTTAGCAGAGTTTTTTGTTAATGAAGCTCGGAAAA
ACAACCATCATTTTAAATCTGAATCTGAAGAGGAGAAAGCATTGATTTAT
CAGTTCAGTCCAATTTATACTGGAAATATTTCTTCATTTCAGCAATGTTAC
ATATTTGATTGAAAGTCTTCAATTTGTTAACAGAGCAAATTTGAATAATTC
CATGATTAAACTGTTAGAATAACTTGCTACTCATGGCAAGATTAGGAAGT
CACAGATTCTTTTCTATAATGTGCCTGGCTCTGATTCTTCATTATGTATGTG
ACTATTTATATAACATTAGATAATTAAATAGTGAGACATAAATAGAGTGC
TTTTTCATGGAAAAGCCTTCTTATATCTGAAGATTGAAAAATAAATTTACT
GAAATACAAAAAAAAAAAAAAA SEQ ID NO: 70

Leucine Proline-Enriched Proteoglycan 1

>gi|21361917|ref|NM_022356.2| Homo sapiens leucine proline-enriched proteoglycan (leprecan) 1 (LEPRE1), mRNA | qPCR forward_primer match [813..836] | qPCR reverse_primer match [894..872] | qPCR probe match [841..870]

GGTGGCGGGTGGCTGGCGGTTCCGTTAGGTCTGAGGGAGCGATGG
CGGTACGCGCGTTGAAGCTGCTGACCACACTGCTGGCTGTCGTGGCCGCT
GCCTCCCAAGCCGAGGTCGAGTCCGAGGCAGGATGGGGCATGGTGACGCC
TGATCTGCTCTTCGCCGAGGGGACCGCAGCCTACGCGCGCGGGGACTGGC
CCGGGGTGGTCCTGAGCATGGAACGGGCGCTGCGCTCCCGGGCAGCCCTC
CGCGCCCTTCGCCTGCGCTGCCGCACCCAGTGTGCCGCCGACTTCCCGTGG
GAGCTGGACCCCGACTGGTCCCCCAGCCCGGCCCAGGCCTCGGGCGCCGC
CGCCCTGCGCGACCTGAGCTTCTTCGGGGGCCTTCTGCGTCGCGCTGCCTG
CCTGCGCCGCTGCCTCGGGCCGCCGGCCGCCCACTCGCTCAGCGAAGAGA
TGGAGCTGGAGTTCCGCAAGCGGAGCCCCTACAACTACCTGCAGGTCGCC
TACTTCAAGATCAACAAGTTGGAGAAAGCTGTTGCTGCAGCACACACCTT
CTTCGTGGGCAATCCTGAGCACATGGAAATGCAGCAGAACCTAGACTATT
ACCAAACCATGTCTGGAGTGAAGGAGGCCGACTTCAAGGATCTTGAGACT
CAACCCCATATGCAAGAATTTCGACTGGGAGTGCGACTCTACTCAGAGGA
ACAGCCACAGGAAGCTGTGCCCCACCTAGAGGCGGCGCTGCAAGAATACT
TTGTGGCCTATGAGGAGTGCCGTGCCCTCTGCGAAGGGCCCTATGACTAC
GATGGCTACAACTACCTTGAGTACAACGCTGACCTCTTCCAGGCCATCAC
AGATCATTACATCCAGGTCCTCAACTGTAAGCAGAACTGTGTCACGGAGC
TTGCTTCCCACCCAAGTCGAGAGAAGCCCTTTGAAGACTTCCTCCCATCGC
ATTATAATTATCTGCAGTTTGCCTACTATAACATTGGGAATTATACACAGG
CTGTTGAATGTGCCAAGACCTATCTTCTCTTCTTCCCCAATGACGAGGTGA
TGAACCAAAATTTGGCCTATTATGCAGCTATGCTTGGAGAAGAACACACC
AGATCCATCGGCCCCCGTGAGAGTGCCAAGGAGTACCGACAGCGAAGCCT
ACTGGAAAAAGAACTGCTTTTCTTCGCTTATGATGTTTTTGGAATTCCCTTT
GTGGATCCGGATTCATGGACTCCAGGAGAAGTGATTCCCAAGAGATTGCA
AGAGAAACAGAAGTCAGAACGGGAAACAGCCGTACGCATCTCCCAGGAG
ATTGGGAACCTTATGAAGGAAATCGAGACCCTTGTGGAAGAGAAGACCA
AGGAGTCACTGGATGTGAGCAGACTGACCCGGGAAGGTGGCCCCCTGCTG
TATGAAGGCATCAGTCTCACCATGAACTCCAAACTCCTGAATGGTTCCCA
GCGGGTGGTGATGGACGGCGTAATCTCTGACCACGAGTGTCAGGAGCTGC
AGAGACTGACCAATGTGGCAGCAACCTCAGGAGATGGCTACCGGGGTCA
GACCTCCCCACATACTCCCAATGAAAAGTTCTATGGTGTCACTGTCTTCAA
AGCCCTCAAGCTGGGGCAAGAAGGCAAAGTTCCTCTGCAGAGTGCCCACC
TGTACTACAACGTGACGGAGAAGGTGCGGCGCATCATGGAGTCCTACTTC
CGCCTGGATACGCCCCTCTACTTTTCCTACTCTCATCTGGTGTGCCGCACT
GCCATCGAAGAGGTCCAGGCAGAGAGGAAGGATGATAGTCATCCAGTCC
ACGTGGACAACTGCATCCTGAATGCCGAGACCCTCGTGTGTGTCAAAGAG
CCCCCAGCCTACACCTTCCGCGACTACAGCGCCATCCTTTACCTAAATGGG
GACTTCGATGGCGGAAACTTTTATTTCACTGAACTGGATGCCAAGACCGT
GACGGCAGAGGTGCAGCCTCAGTGTGGAAGAGCCGTGGGATTCTCTTCAG
GCACTGAAAACCCACATGGAGTGAAGGCTGTCACCAGGGGGCAGCGCTGT
GCCATCGCCCTGTGGTTCACCCTGGACCCTCGACACAGCGAGCGGGTGAG
AGCAGCTCGAGCGGGTGAGAGCAGCTGGTGCTGTGGTGACCCGTTCCCAG

AGCGCCCTTGGTTTGCCTTTCTCTTCCCCAAATCCCATTGCCAGTGGCTGA
GACACGAAAGGAGCACTTGGGACACCAGCTCCAACGCCCTGTCATTATGG
TCACATTGCCTTGTCCTCCCTGGGCCTGCTGTGAACGGGATCCAGGTGGGG
AAAGAGGTCAAGACAGGGAGCGATGCTGAGTTCTTGGTTCCCTCCTTGGG
CCCCACTTCAGCTGTCCTTTTCCAGAGAGTAGGACCTGCTGGGAAGGAGA
TGAGCCTGGGGCCATTAAGGAACCTTCCTTGTCCCCTGGGAAGTAGCAGC
TGAGAGATAGCGAGTGTCTGGAGCGGAGGCCTCTCTGAATGGGCAGGGGT
TTGTCCTTGCAGGACAGGGTGCAGGCAGATGACCTGGTGAAGATGCTCTT
CAGCCCAGAAGAGATGGTCCTCTCCCAGGAGCAGCCCCTGGATGCCCAGC
AGGGCCCCCCCGAACCTGCACAAGAGTCTCTCTCAGGCAGTGAATCGAAG
CCCAAGGATGAGCTATGACAGCGTCCAGGTCAGACGGATGGGTGACTAGA
CCCATGGAGAGGAACTCTTCTGCACTCTGAGCTGGCCAGCCCCTCGGGGC
TGCAGAGCAGTGAGCCTACATCTGCCACTCAGCCGAGGGGACCCTGCTCA
CAGCCTTCTACATGGTGCTACTGCTCTTGGAGTGGACATGACCAGACACC
GCACCCCCTGGATCTGGCTGAGGGCTCAGGACACAGGCCCAGCCACCCCC
AGGGGCCTCCACAGGCCGCTGCATAACAGCGATACAGTACTTAAGTGTCT
GTGTAGACAACCAAAGAATAAATGATTCATGGTTTTTTTT

SEQ ID NO: 71

Cystatin S

>gi|19882254|ref|NM_001899.2| Homo sapiens cystatin S (CST4), mRNA | qPCR forward_primer match [343..361] | qPCR reverse_primer match [434..411] | qPCR probe match [382..410]

GGCTCTCACCCTCCTCTCCTGCAGCTCCAGCTTTGTGCTCTGCCTCT
GAGGAGACCATGGCCCGGCCTCTGTGTACCCTGCTACTCCTGATGGCTACC
CTGGCTGGGGCTCTGGCCTCGAGCTCCAAGGAGGAGAATAGGATAATCCC
AGGTGGCATCTATGATGCAGACCTCAATGATGAGTGGGTACAGCGTGCCC
TTCACTTCGCCATCAGCGAGTACAACAAGGCCACCGAAGATGAGTACTAC
AGACGCCCGCTGCAGGTGCTGCGAGCCAGGGAGCAGACCTTTGGGGGGGT
GAATTACTTCTTCGACGTAGAGGTGGGCCGCACCATATGTACCAAGTCCC
AGCCCAACTTGGACACCTGTGCCTTCCATGAACAGCCAGAACTGCAGAAG
AAACAGTTGTGCTCTTTCGAGATCTACGAAGTTCCCTGGGAGGACAGAAT
GTCCCTGGTGAATTCCAGGTGTCAAGAAGCCTAGGGGTCTGTGCCAGGCC
AGTCACACCGACCACCACCCACTCCCACCCACTGTAGTGCTCCCACCCCTG
GACTGGTGGCCCCCACCCTGCGGGAGGCCTCCCCATGTGCCTGTGCCAAG
AGACAGACAGAGAAGGCTGCAGGAGTCCTTTGTTGCTCAGCAGGGCGCTC
TGCCCTCCCTCCTTCCTTCTTGCTTCTAATAGACCTGGTACATGGTACACAC
ACCCCCACCTCCTGCAATTAAACAGTAGCATCGCC SEQ ID NO: 72

Secreted Frizzle-Related Protein 4

>gi|8400733|ref|NM_003014.2| Homo sapiens secreted frizzled-related protein 4 (SFRP4), mRNA | qPCR assay_on_demand_context match [1079..1103]

GGCGGGTTCGCGCCCCGAAGGCTGAGAGCTGGCGCTGCTCGTGCCC
TGTGTGCCAGACGGCGGAGCTCCGCGGCCGGACCCCGCGGCCCCGCTTTG
CTGCCGACTGGAGTTTGGGGGAAGAAACTCTCCTGCGCCCCAGAAGATTT
CTTCCTCGGCGAAGGGACAGCGAAAGATGAGGGTGGCAGGAAGAGAAGG
CGCTTTCTGTCTGCCGGGGTCGCAGCGCGAGAGGGCAGTGCCATGTTCCTC

TCCATCCTAGTGGCGCTGTGCCTGTGGCTGCACCTGGCGCTGGGCGTGCGC
GGCGCGCCCTGCGAGGCGGTGCGCATCCCTATGTGCCGGCACATGCCCTG
GAACATCACGCGGATGCCCAACCACCTGCACCACAGCACGCAGGAGAAC
GCCATCCTGGCCATCGAGCAGTACGAGGAGCTGGTGGACGTGAACTGCAG
CGCCGTGCTGCGCTTCTTCTTCTGTGCCATGTACGCGCCCATTTGCACCCT
GGAGTTCCTGCACGACCCTATCAAGCCGTGCAAGTCGGTGTGCCAACGCG
CGCGCGACGACTGCGAGCCCCTCATGAAGATGTACAACCACAGCTGGCCC
GAAAGCCTGGCCTGCGACGAGCTGCCTGTCTATGACCGTGGCGTGTGCAT
TTCGCCTGAAGCCATCGTCACGGACCTCCCGGAGGATGTTAAGTGGATAG
ACATCACACCAGACATGATGGTACAGGAAAGGCCTCTTGATGTTGACTGT
AAACGCCTAAGCCCCGATCGGTGCAAGTGTAAAAAGGTGAAGCCAACTTT
GGCAACGTATCTCAGCAAAAACTACAGCTATGTTATTCATGCCAAAATAA
AAGCTGTGCAGAGGAGTGGCTGCAATGAGGTCACAACGGTGGTGGATGTA
AAAGAGATCTTCAAGTCCTCATCACCCATCCCTCGAACTCAAGTCCCGCTC
ATTACAAATTCTTCTTGCCAGTGTCCACACATCCTGCCCCATCAAGATGTT
CTCATCATGTGTTACGAGTGGCGTTCAAGGATGATGCTTCTTGAAAATTGC
TTAGTTGAAAAATGGAGAGATCAGCTTAGTAAAAGATCCATACAGTGGGA
AGAGAGGCTGCAGGAACAGCGGAGAACAGTTCAGGACAAGAAGAAAACA
GCCGGGCGCACCAGTCGTAGTAATCCCCCCAAACCAAAGGGAAAGCCTCC
TGCTCCCAAACCAGCCAGTCCCAAGAAGAACATTAAAACTAGGAGTGCCC
AGAAGAGAACAAACCCGAAAAGAGTGTGAGCTAACTAGTTTCCAAAGCG
GAGACTTCCGACTTCCTTACAGGATGAGGCTGGGCATTGCCTGGGACAGC
CTATGTAAGGCCATGTGCCCCTTGCCCTAACAACTCACTGCAGTGCTCTTC
ATAGACACATCTTGCAGCATTTTTCTTAAGGCTATGCTTCAGTTTTTCTTTG
TAAGCCATCACAAGCCATAGTGGTAGGTTTGCCCTTTGGTACAGAAGGTG
AGTTAAAGCTGGTGGAAAAGGCTTATTGCATTGCATTCAGAGTAACCTGT
GTGCATACTCTAGAAGAGTAGGGAAAATAATGCTTGTTACAATTCGACCT
AATATGTGCATTGTAAAATAAATGCCATATTTCAAACAAAACACGTAATT
TTTTTACAGTATGTTTTATTACCTTTTGATATCTGTTGTTGCAATGTTAGTG
ATGTTTTAAAATGTGATGAAAATATAATGTTTTTAAGAAGGAACAGTAGT
GGAATGAATGTTAAAAGATCTTTATGTGTTTATGGTCTGCAGAAGGATTTT
TGTGATGAAAGGGGATTTTTTGAAAAATTAGAGAAGTAGCATATGGAAAA
TTATAATGTGTTTTTTTTACCAATGACTTCAGTTTCTGTTTTTAGCTAGAAAC
TTAAAAAACAAAAATAATAATAAAGAAAAATAAATAAAAAGGAGAGGCAG
ACAATGTCTGGATTCCTGTTTTTTGGTTACCTGATTTCCATGATCATGATGC
TTCTTGTCAACACCCTCTTAAGCAGCACCAGAAACAGTGAGTTTGTCTGTA
CCATTAGGAGTTAGGTACTAATTAGTTGGCTAATGCTCAAGTATTTTATAC
CCACAAGAGAGGTATGTCACTCATCTTACTTCCCAGGACATCCACCCTGA
GAATAATTTGACAAGCTTAAAAATGGCCTTCATGTGAGTGCCAAATTTTGT
TTTTCTTCATTTAAATATTTTCTTTGCCTAAATACATGTGAGAGGAGTTAA
ATATAAATGTACAGAGAGGAAAGTTGAGTTCCACCTCTGAAATGAGAATT
ACTTGACAGTTGGGATACTTTAATCAGAAAAAAAGAACTTATTTGCAGCA
TTTTATCAACAAATTTCATAATTGTGGACAATTGGAGGCATTTATTTTAAA
AAACAATTTTATTGGCCTTTTGCTAACACAGTAAGCATGTATTTTATAAGG
CATTCAATAAATGCACAACGCCCAAAGGAAATAAAATCCTATCTAATCCT
ACTCTCCACTACACAGAGGTAATCACTATTAGTATTTTGGCATATTATTCT
CCAGGTGTTTGCTTATGCACTTATAAAATGATTTGAACAAATAAAACTAG
GAACCTGTATACATGTGTTTCATAACCTGCCTCCTTTGCTTGGCCCTTTATT
GAGATAAGTTTTCCTGTCAAGAAAGCAGAAACCATCTCATTTCTAACAGC
TGTGTTATATTCCATAGTATGCATTACTCAACAAACTGTTGTGCTATTGGA

TACTTAGGTGGTTTCTTCACTGACAATACTGAATAAACATCTCACCGGAAT
TC
SEQ ID NO: 73

Asporin

>gi|41350213|ref|NM_017680.3| Homo sapiens asporin (LRR class 1) (ASPN), mRNA | qPCR forward_primer match [798..823] | qPCR reverse_primer match [934..912] | qPCR probe match [842..875]

AGTACTAACATGGACTAATCTGTGGGAGCAGTTTATTCCAGTATCA
CCCAGGGTGCAGCCACACCAGGACTGTGTTGAAGGGTGTTTTTTTTCTTTT
AAATGTAATACCTCCTCATCTTTTCTTCTTACACAGTGTCTGAGAACATTT
ACATTATAGATAAGTAGTACATGGTGGATAACTTCTACTTTTAGGAGGACT
ACTCTCTTCTGACAGTCCTAGACTGGTCTTCTACACTAAGACACCATGAAG
GAGTATGTGCTCCTATTATTCCTGGCTTTGTGCTCTGCCAAACCCTTCTTTA
GCCCTTCACACATCGCACTGAAGAATATGATGCTGAAGGATATGGAAGAC
ACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAGGA
CAACTCTCTTTTTCCAACAAGAGAGCCAAGAAGCCATTTTTTTCCATTTGA
TCTGTTTCCAATGTGTCCATTTGGATGTCAGTGCTATTCACGAGTTGTACA
TTGCTCAGATTTAGGTTTGACCTCAGTCCCAACCAACATTCCATTTGATAC
TCGAATGCTTGATCTTCAAAACAATAAAATTAAGGAAATCAAAGAAAATG
ATTTTAAAGGACTCACTTCACTTTATGGTCTGATCCTGAACAACAACAAGC
TAACGAAGATTCACCCAAAAGCCTTTCTAACCACAAAGAAGTTGCGAAGG
CTGTATCTGTCCCACAATCAACTAAGTGAAATACCACTTAATCTTCCCAAA
TCATTAGCAGAACTCAGAATTCATGAAAATAAAGTTAAGAAAATACAAAA
GGACACATTCAAAGGAATGAATGCTTTACACGTTTTGGAAATGAGTGCAA
ACCCTCTTGATAATAATGGGATAGAGCCAGGGGCATTTGAAGGGGTGACG
GTGTTCCATATCAGAATTGCAGAAGCAAAACTGACCTCAGTTCCTAAAGG
CTTACCACCAACTTTATTGGAGCTTCACTTAGATTATAATAAAATTTCAAC
AGTGGAACTTGAGGATTTTAAACGATACAAAGAACTACAAAGGCTGGGCC
TAGGAAACAACAAAATCACAGATATCGAAAATGGGAGTCTTGCTAACATA
CCACGTGTGAGAGAAATACATTTGGAAAACAATAAACTAAAAAAAATCCC
TTCAGGATTACCAGAGTTGAAATACCTCCAGATAATCTTCCTTCATTCTAA
TTCAATTGCAAGAGTGGGAGTAAATGACTTCTGTCCAACAGTGCCAAAGA
TGAAGAAATCTTTATACAGTGCAATAAGTTTATTCAACAACCCGGTGAAA
TACTGGGAAATGCAACCTGCAACATTTCGTTGTGTTTTGAGCAGAATGAGT
GTTCAGCTTGGGAACTTTGGAATGTAATAATTAGTAATTGGTAATGTCCAT
TTAATATAAGATTCAAAAATCCCTACATTTGGAATACTTGAACTCTATTAA
TAATGGTAGTATTATATATACAAGCAAATATCTATTCTCAAGTGGTAAGTC
CACTGACTTATTTTATGACAAGAAATTTCAACGGAATTTTGCCAAACTATT
GATACATAAGGGTTGAGAGAAACAAGCATCTATTGCAGTTTCTTTTTGCGT
ACAAATGATCTTACATAAATCTCATGCTTGACCATTCCTTTCTTCATAACA
AAAAAGTAAGATATTCGGTATTTAACACTTTGTTATCAAGCATATTTTAAA
AAGAACTGTACTGTAAATGGAATGCTTGACTTAGCAAAATTTGTGCTCTTT
CATTTGCTGTTAGAAAAACAGAATTAACAAAGACAGTAATGTGAAGAGTG
CATTACACTATTCTTATTCTTTAGTAACTTGGGTAGTACTGTAATATTTTTA
ATCATCTTAAAGTATGATTTGATATAATCTTATTGAAATTACCTTATCATG
TCTTAGAGCCCGTCTTTATGTTTAAAACTAATTTCTTAAAATAAAGCCTTC
AGTAAATGTTCATTACCAACTTGATAAATGCTACTCATAAGAGCTGGTTTG
GGGCTATAGCATATGCTTTTTTTTTTTTAATTATTACCTGATTTAAAAATCT

CTGTAAAAACGTGTAGTGTTTCATAAAATCTGTAACTCGCATTTTAATGAT
CCGCTATTATAAGCTTTTAATAGCATGAAAATTGTTAGGCTATATAACATT
GCCACTTCAACTCTAAGGAATATTTTTGAGATATCCCTTTGGAAGACCTTG
CTTGGAAGAGCCTGGACACTAACAATTCTACACCAAATTGTCTCTTCAAAT
ACGTATGGACTGGATAACTCTGAGAAACACATCTAGTATAACTGAATAAG
CAGAGCATCAAATTAAACAGACAGAAACCGAAAGCTCTATATAAATGCTC
AGAGTTCTTTATGTATTTCTTATTGGCATTCAACATATGTAAAATCAGAAA
ACAGGGAAATTTTCATTAAAAATATTGGTTTGAAATAAAAAAAAAAAAAA

SEQ ID NO: 74

Cell Growth Regulator with EF Hand Domain 1

>gi|33589823|ref|NM_006569.2| Homo sapiens cell growth regulator with EF hand domain 1 (CGREF1), mRNA | qPCR forward_primer match [378..394] | qPCR reverse_primer match [455..431] | qPCR probe match [396..415]

CGCGCAGCCCCTCCGGCCGCGGGCGCAGCGGGGGCGCTGGTGGAG
CTGCGAAGGGCCAGGTCCGGCGGGCGGGGCGGCGGCTGGCACTGGCTCC
GGACTCTGCCCGGCCAGGGCGGCGGCTCCAGCCGGGAGGGCGACGTGGA
GCGGCCACGTGGAGCGGCCCGGGGGAGGCTGGCGGCGGGAGGCGAGGCG
CGGGCGGCGCAGCAGCCAGGAGCGCCCACGGAGCTGGACCCCCAGAGCC
GCGCGGCGCCGCAGCAGTTCCAGGAAGGATGTTACCTTTGACGATGACAG
TGTTAATCCTGCTGCTGCTCCCCACGGGTCAGGCTGCCCCAAAGGATGGA
GTCACAAGGCCAGACTCTGAAGTGCAGCATCAGCTCCTGCCCAACCCCTT
CCAGCCAGGCCAGGAGCAGCTCGGACTTCTGCAGAGCTACCTAAAGGGAC
TAGGAAGGACAGAAGTGCAACTGGAGCATCTGAGCCGGGAGCAGGTTCT
CCTCTACCTCTTTGCCCTCCATGACTATGACCAGAGTGGACAGCTGGATGG
CCTGGAGCTGCTGTCCATGTTGACAGCTGCTCTGGCCCCTGGAGCTGCCAA
CTCTCCTACCACCAACCCGGTGATATTGATAGTGGACAAAGTGCTCGAGA
CGCAGGACCTGAATGGGGATGGGCTCATGACCCCTGCTGAGCTCATCAAC
TTCCCGGGAGTAGCCCTCAGGCACGTGGAGCCCGGAGAGCCCCTTGCTCC
ATCTCCTCAGGAGCCACAAGCTGTTGGAAGGCAGTCCCTATTAGCTAAAA
GCCCATTAAGACAAGAAACACAGGAAGCCCCTGGTCCCAGAGAAGAAGC
AAAGGGCCAGGTAGAGGCCAGAAGGGAGTCTTTGGATCCTGTCCAGGAG
CCTGGGGGCCAGGCAGAGGCTGATGGAGATGTTCCAGGGCCCAGAGGGG
AAGCTGAGGGCCAGGCAGAGGCTAAAGGAGATGCCCCTGGGCCCAGAGG
GGAAGCTGGGGGCCAGGCAGAGGCTGAAGGAGATGCCCCCGGGCCCAGA
GGGGAAGCTGGGGGCCAGGCAGAGGCCAGGGAGAATGGAGAGGAGGCC
AAGGAACTTCCAGGGGAAACACTGGAGTCTAAGAACACCCAAAATGACTT
TGAGGTGCACATTGTTCAAGTGGAGAATGATGAGATCTAGATCTTGAAGA
TACAGGTACCCCACGAAGTCTCAGTGCCAGAACATAAGCCCTGAAGTGGG
CAGGGGAAATGTACGCTGGGACAAGGACCATCTCTGTGCCCCCTGTCTGG
TCCCAGTAGGTATCAGGTCTTTCTGTGCAGCTCAGGGAGACCCTAAGTTAA
GGGGCAGATTACCAATAAAGAACTGAATGAATTCATCCCCCCGGGCCACC
TCTCTACCCGTCCAGCCTGCCCAGACCCTCTCAGAGGAACGGGGTTGGGG
ACCGAAAGGACAGGGATGCCGCCTGCCCAGTGTTTCTGGGCCTCACGGTG
CTCCGGCAGCAGAGCGCATGGTGCTAGCCATGGCCGGCTGCAGAGGACCC
AGTGAGGAAAGCTCAGTCTATCCCTGGGCCCCAAACCCTCACCGGTTCCC
CCTCACCTGGTGTTCAGACACCCCATGCTCTCCTGCAGCTCAGGGCAGGTG
ACCCCATCCCCAGTAATATTAATCATCACTAGAACTTTTTGAGAGCCTTGT
ACACATCAGGCATCATGCTGGGCATTTTATATATGATTTTATCCTCACAAT

AATTCTGTAGCCAAGCAGAATTGGTTCCATTTGACAGATGAAGAAATTGA
GGCAGATTGCGTTAAGTGCTGTACCCTAAGGTGATATGCAGCTAATTAAA
TGGCAGATTTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
SEQ ID NO: 75

Kallikrein 10, Transcript Variant 1

>gi|22208981|ref|NM_002776.3| Homo sapiens kallikrein 10 (KLK10), transcript variant 1, mRNA | qPCR forward_primer match [851..874] | qPCR reverse_primer match [950..931] | qPCR probe match [890..914]

CATCCTGCCACCCCTAGCCTTGCTGGGGACGTGAACCCTCTCCCCG
CGCCTGGGAAGCCTTCTTGGCACCGGGACCCGGAGAATCCCCACGGAAGC
CAGTTCCAAAAGGGATGAAAAGGGGGCGTTTCGGGCACTGGGAGAAGCC
TGTATTCCAGGGCCCCTCCCAGAGCAGGAATCTGGGACCCAGGAGTGCCA
GCCTCACCCACGCAGATCCTGGCCATGAGAGCTCCGCACCTCCACCTCTCC
GCCGCCTCTGGCGCCCGGGCTCTGGCGAAGCTGCTGCCGCTGCTGATGGC
GCAACTCTGGGCCGCAGAGGCGGCGCTGCTCCCCCAAAACGACACGCGCT
TGGACCCCGAAGCCTATGGCTCCCCGTGCGCGCGCGGCTCGCAGCCCTGG
CAGGTCTCGCTCTTCAACGGCCTCTCGTTCCACTGCGCGGGTGTCCTGGTG
GACCAGAGTTGGGTGCTGACGGCCGCGCACTGCGGAAACAAGCCACTGTG
GGCTCGAGTAGGGGATGACCACCTGCTGCTTCTTCAGGGAGAGCAGCTCC
GCCGGACCACTCGCTCTGTTGTCCATCCCAAGTACCACCAGGGCTCAGGC
CCCATCCTGCCAAGGCGAACGGATGAGCACGATCTCATGTTGCTGAAGCT
GGCCAGGCCCGTAGTGCTGGGGCCCCGCGTCCGGGCCCTGCAGCTTCCCT
ACCGCTGTGCTCAGCCCGGAGACCAGTGCCAGGTTGCTGGCTGGGGCACC
ACGGCCGCCCGGAGAGTGAAGTACAACAAGGGCCTGACCTGCTCCAGCAT
CACTATCCTGAGCCCTAAAGAGTGTGAGGTCTTCTACCCTGGCGTGGTCAC
CAACAACATGATATGTGCTGGACTGGACCGGGGCCAGGACCCTTGCCAGA
GTGACTCTGGAGGCCCCCTGGTCTGTGACGAGACCCTCCAAGGCATCCTCT
CGTGGGGTGTTTACCCCTGTGGCTCTGCCCAGCATCCAGCTGTCTACACCC
AGATCTGCAAATACATGTCCTGGATCAATAAAGTCATACGCTCCAACTGA
TCCAGATGCTACGCTCCAGCTGATCCAGATGTTATGCTCCTGCTGATCCAG
ATGCCCAGAGGCTCCATCGTCCATCCTCTTCCTCCCCAGTCGGCTGAACTC
TCCCCTTGTCTGCACTGTTCAAACCTCTGCCGCCCTCCACACCTCTAAACA
TCTCCCCTCTCACCTCATTCCCCCACCTATCCCCATTCTCTGCCTGTACTGA
AGCTGAAATGCAGGAAGTGGTGGCAAAGGTTTATTCCAGAGAAGCCAGG
AAGCCGGTCATCACCCAGCCTCTGAGAGCAGTTACTGGGGTCACCCAACC
TGACTTCCTCTGCCACTCCCTGCTGTGTGACTTTGGGCAAGCCAAGTGCCC
TCTCTGAACCTCAGTTTCCTCATCTGCAAAATGGGAACAATGACGTGCCTA
CCTCTTAGACATGTTGTGAGGAGACTATGATATAACATGTGTATGTAAATC
TTCATGGTGATTGTCATGTAAGGCTTAACACAGTGGGTGGTGAGTTCTGAC
TAAAGGTTACCTGTTGTCGTGA SEQ ID NO: 76

Kallikrein 10 Transcript Variant 2

>gi|22208983|ref|NM_145888.1| Homo sapiens kallikrein 10 (KLK10), transcript variant 2, mRNA | qPCR forward_primer match [714..737] | qPCR reverse_primer match [813..794] | qPCR probe match [753..777]

ACCAGCGGCAGACCACAGGCAGGGCAGAGGCACGTCTGGGTCCCC
TCCCTCCTTCCTATCGGCGACTCCCAGGATCCTGGCCATGAGAGCTCCGCA
CCTCCACCTCTCCGCCGCCTCTGGCGCCCGGGCTCTGGCGAAGCTGCTGCC
GCTGCTGATGGCGCAACTCTGGGCCGCAGAGGCGGCGCTGCTCCCCCAAA
ACGACACGCGCTTGGACCCCGAAGCCTATGGCTCCCCGTGCGCGCGCGGC
TCGCAGCCCTGGCAGGTCTCGCTCTTCAACGGCCTCTCGTTCCACTGCGCG
GGTGTCCTGGTGGACCAGAGTTGGGTGCTGACGGCCGCGCACTGCGGAAA
CAAGCCACTGTGGGCTCGAGTAGGGGATGACCACCTGCTGCTTCTTCAGG
GAGAGCAGCTCCGCCGGACCACTCGCTCTGTTGTCCATCCCAAGTACCAC
CAGGGCTCAGGCCCCATCCTGCCAAGGCGAACGGATGAGCACGATCTCAT
GTTGCTGAAGCTGGCCAGGCCCGTAGTGCTGGGGCCCCGCGTCCGGGCCC
TGCAGCTTCCCTACCGCTGTGCTCAGCCCGGAGACCAGTGCCAGGTTGCTG
GCTGGGGCACCACGGCCGCCCGGAGAGTGAAGTACAACAAGGGCCTGAC
CTGCTCCAGCATCACTATCCTGAGCCCTAAAGAGTGTGAGGTCTTCTACCC
TGGCGTGGTCACCAACAACATGATATGTGCTGGACTGGACCGGGGCCAGG
ACCCTTGCCAGAGTGACTCTGGAGGCCCCCTGGTCTGTGACGAGACCCTC
CAAGGCATCCTCTCGTGGGGTGTTTACCCCTGTGGCTCTGCCCAGCATCCA
GCTGTCTACACCCAGATCTGCAAATACATGTCCTGGATCAATAAAGTCAT
ACGCTCCAACTGATCCAGATGCTACGCTCCAGCTGATCCAGATGTTATGCT
CCTGCTGATCCAGATGCCCAGAGGCTCCATCGTCCATCCTCTTCCTCCCCA
GTCGGCTGAACTCTCCCCTTGTCTGCACTGTTCAAACCTCTGCCGCCCTCC
ACACCTCTAAACATCTCCCCTCTCACCTCATTCCCCCACCTATCCCCATTCT
CTGCCTGTACTGAAGCTGAAATGCAGGAAGTGGTGGCAAAGGTTTATTCC
AGAGAAGCCAGGAAGCCGGTCATCACCCAGCCTCTGAGAGCAGTTACTGG
GGTCACCCAACCTGACTTCCTCTGCCACTCCCTGCTGTGTGACTTTGGGCA
AGCCAAGTGCCCTCTCTGAACCTCAGTTTCCTCATCTGCAAAATGGGAACA
ATGACGTGCCTACCTCTTAGACATGTTGTGAGGAGACTATGATATAACAT
GTGTATGTAAATCTTCATGGTGATTGTCATGTAAGGCTTAACACAGTGGGT
GGTGAGTTCTGACTAAAGGTTACCTGTTGTCGTGA SEQ ID NO: 77

Tissue Inhibitor of Metalloproteinase 1

>gi|4507508|ref|NM_003254.1| Homo sapiens tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1), mRNA | qPCR forward_primer match [221..241] | qPCR reverse_primer match [359..340] | qPCR probe match [251..283]

AGGGGCCTTAGCGTGCCGCATCGCCGAGATCCAGCGCCCAGAGAG
ACACCAGAGAACCCACCATGGCCCCCTTTGAGCCCCTGGCTTCTGGCATCC
TGTTGTTGCTGTGGCTGATAGCCCCCAGCAGGGCCTGCACCTGTGTCCCAC
CCCACCCACAGACGGCCTTCTGCAATTCCGACCTCGTCATCAGGGCCAAG
TTCGTGGGGACACCAGAAGTCAACCAGACCACCTTATACCAGCGTTATGA
GATCAAGATGACCAAGATGTATAAAGGGTTCCAAGCCTTAGGGGATGCCG
CTGACATCCGGTTCGTCTACACCCCCGCCATGGAGAGTGTCTGCGGATACT
TCCACAGGTCCCACAACCGCAGCGAGGAGTTTCTCATTGCTGGAAAACTG
CAGGATGGACTCTTGCACATCACTACCTGCAGTTTCGTGGCTCCCTGGAAC
AGCCTGAGCTTAGCTCAGCGCCGGGGCTTCACCAAGACCTACACTGTTGG
CTGTGAGGAATGCACAGTGTTTCCCTGTTTATCCATCCCCTGCAAACTGCA
GAGTGGCACTCATTGCTTGTGGACGGACCAGCTCCTCCAAGGCTCTGAAA

AGGGCTTCCAGTCCCGTCACCTTGCCTGCCTGCCTCGGGAGCCAGGGCTGT
GCACCTGGCAGTCCCTGCGGTCCCAGATAGCCTGAATCCTGCCCGGAGTG
GAACTGAAGCCTGCACAGTGTCCACCCTGTTCCCACTCCCATCTTTCTTCC
GGACAATGAAATAAAGAGTTACCACCCAGC SEQ ID NO: 78

Secreted Protein, Acidic, Cysteine-Rich

>gi|48675809|ref|NM_003118.2| Homo sapiens secreted protein, acidic, cysteine-rich (osteonectin) (SPARC), mRNA | qPCR forward_primer match [788..810] | qPCR reverse_primer match [915..898] | qPCR probe match [818..839]

GTTGCCTGTCTCTAAACCCCTCCACATTCCCGCGGTCCTTCAGACTG
CCCGGAGAGCGCGCTCTGCCTGCCGCCTGCCTGCCTGCCACTGAGGGTTCC
CAGCACCATGAGGGCCTGGATCTTCTTTCTCCTTTGCCTGGCCGGGAGGGC
CTTGGCAGCCCCTCAGCAAGAAGCCCTGCCTGATGAGACAGAGGTGGTGG
AAGAAACTGTGGCAGAGGTGACTGAGGTATCTGTGGGAGCTAATCCTGTC
CAGGTGGAAGTAGGAGAATTTGATGATGGTGCAGAGGAAACCGAAGAGG
AGGTGGTGGCGGAAAATCCCTGCCAGAACCACCACTGCAAACACGGCAA
GGTGTGCGAGCTGGATGAGAACAACACCCCCATGTGCGTGTGCCAGGACC
CCACCAGCTGCCCAGCCCCCATTGGCGAGTTTGAGAAGGTGTGCAGCAAT
GACAACAAGACCTTCGACTCTTCCTGCCACTTCTTTGCCACAAAGTGCACC
CTGGAGGGCACCAAGAAGGGCCACAAGCTCCACCTGGACTACATCGGGCC
TTGCAAATACATCCCCCCTTGCCTGGACTCTGAGCTGACCGAATTCCCCCT
GCGCATGCGGGACTGGCTCAAGAACGTCCTGGTCACCCTGTATGAGAGGG
ATGAGGACAACAACCTTCTGACTGAGAAGCAGAAGCTGCGGGTGAAGAA
GATCCATGAGAATGAGAAGCGCCTGGAGGCAGGAGACCACCCCGTGGAG
CTGCTGGCCCGGGACTTCGAGAAGAACTATAACATGTACATCTTCCCTGTA
CACTGGCAGTTCGGCCAGCTGGACCAGCACCCCATTGACGGGTACCTCTC
CCACACCGAGCTGGCTCCACTGCGTGCTCCCCTCATCCCCATGGAGCATTG
CACCACCCGCTTTTTCGAGACCTGTGACCTGGACAATGACAAGTACATCG
CCCTGGATGAGTGGGCCGGCTGCTTCGGCATCAAGCAGAAGGATATCGAC
AAGGATCTTGTGATCTAAATCCACTCCTTCCACAGTACCGGATTCTCTCTT
TAACCCTCCCCTTCGTGTTTCCCCCAATGTTTAAAATGTTTGGATGGTTTGT
TGTTCTGCCTGGAGACAAGGTGCTAACATAGATTTAAGTGAATACATTAA
CGGTGCTAAAAATGAAAATTCTAACCCAAGACATGACATTCTTAGCTGTA
ACTTAACTATTAAGGCCTTTTCCACACGCATTAATAGTCCCATTTTTCTCTT
GCCATTTGTAGCTTTGCCCATTGTCTTATTGGCACATGGGTGGACACGGAT
CTGCTGGGCTCTGCCTTAAACACACATTGCAGCTTCAACTTTTCTCTTTAGT
GTTCTGTTTGAAACTAATACTTACCGAGTCAGACTTTGTGTTCATTTCATTT
CAGGGTCTTGGCTGCCTGTGGGCTTCCCCAGGTGGCCTGGAGGTGGGCAA
AGGGAAGTAACAGACACACGATGTTGTCAAGGATGGTTTTGGGACTAGAG
GCTCAGTGGTGGGAGAGATCCCTGCAGAACCCACCAACCAGAACGTGGTT
TGCCTGAGGCTGTAACTGAGAGAAAGATTCTGGGGCTGTGTTATGAAAAT
ATAGACATTCTCACATAAGCCCAGTTCATCACCATTTCCTCCTTTACCTTTC
AGTGCAGTTTCTTTTCACATTAGGCTGTTGGTTCAAACTTTTGGGAGCACG
GACTGTCAGTTCTCTGGGAAGTGGTCAGCGCATCCTGCAGGGCTTCTCCTC
CTCTGTCTTTTGGAGAACCAGGGCTCTTCTCAGGGGCTCTAGGGACTGCCA
GGCTGTTTCAGCCAGGAAGGCCAAAATCAAGAGTGAGATGTAGAAAGTTG
TAAAATAGAAAAAGTGGAGTTGGTGAATCGGTTGTTCTTTCCTCACATTTG
GATGATTGTCATAAGGTTTTTAGCATGTTCCTCCTTTTCTTCACCCTCCCCT

TTTTTCTTCTATTAATCAAGAGAAACTTCAAAGTTAATGGGATGGTCGGAT
CTCACAGGCTGAGAACTCGTTCACCTCCAAGCATTTCATGAAAAAGCTGC
TTCTTATTAATCATACAAACTCTCACCATGATGTGAAGAGTTTCACAAATC
CTTCAAAATAAAAAGTAATGACTTAGAAACTGCCTTCCTGGGTGATTTGC
ATGTGTCTTAGTCTTAGTCACCTTATTATCCTGACACAAAAACACATGAGC
ATACATGTCTACACATGACTACACAAATGCAAACCTTTGCAAACACATTA
TGCTTTTGCACACACACACCTGTACACACACACCGGCATGTTTATACACAG
GGAGTGTATGGTTCCTGTAAGCACTAAGTTAGCTGTTTTCATTTAATGACC
TGTGGTTTAACCCTTTTGATCACTACCACCATTATCAGCACCAGACTGAGC
AGCTATATCCTTTTATTAATCATGGTCATTCATTCATTCATTCACAAA
ATATTTATGATGTATTTACTCTGCACCAGGTCCCATGCCAAGCACTGGGGA
CACAGTTATGGCAAAGTAGACAAAGCATTTGTTCATTTGGAGCTTAGAGT
CCAGGAGGAATACATTAGATAATGACACAATCAAATATAAATTGCAAGAT
GTCACAGGTGTGATGAAGGGAGAGTAGGAGAGACCATGAGTATGTGTAA
CAGGAGGACACAGCATTATTCTAGTGCTGTACTGTTCCGTACGGCAGCCA
CTACCCACATGTAACTTTTTAAGATTTAAATTTAAATTAGTTAACATTCAA
AACGCAGCTCCCCAATCACACTAGCAACATTTCAAGTGCTTGAGAGCCAT
GCATGATTAGTGGTTACCCTATTGAATAGGTCAGAAGTAGAATCTTTTCAT
CATCACAGAAAGTTCTATTGGACAGTGCTCTTCTAGATCATCATAAGACTA
CAGAGCACTTTTCAAAGCTCATGCATGTTCATCATGTTAGTGTCGTATTTT
GAGCTGGGGTTTTGAGACTCCCCTTAGAGATAGAGAAACAGACCCAAGAA
ATGTGCTCAATTGCAATGGGCCACATACCTAGATCTCCAGATGTCATTTCC
CCTCTCTTATTTTAAGTTATGTTAAGATTACTAAAACAATAAAAGCTCCTA
AAAAATCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 79

Transforming Growth Factor, Beta-Induced

>gi|4507466|ref|NM_000358.1| Homo sapiens transforming growth factor, beta-induced, 68kDa (TGFBI), mRNA | qPCR assay_on_demand_context match [170..194]

GCTTGCCCGTCGGTCGCTAGCTCGCTCGGTGCGCGTCGTCCCGCTCC
ATGGCGCTCTTCGTGCGGCTGCTGGCTCTCGCCCTGGCTCTGGCCCTGGGC
CCCGCCGCGACCCTGGCGGGTCCCGCCAAGTCGCCCTACCAGCTGGTGCT
GCAGCACAGCAGGCTCCGGGGCCGCCAGCACGGCCCCAACGTGTGTGCTG
TGCAGAAGGTTATTGGCACTAATAGGAAGTACTTCACCAACTGCAAGCAG
TGGTACCAAAGGAAAATCTGTGGCAAATCAACAGTCATCAGCTACGAGTG
CTGTCCTGGATATGAAAAGGTCCCTGGGGAGAAGGGCTGTCCAGCAGCCC
TACCACTCTCAAACCTTTACGAGACCCTGGGAGTCGTTGGATCCACCACCA
CTCAGCTGTACACGGACCGCACGGAGAAGCTGAGGCCTGAGATGGAGGG
GCCCGGCAGCTTCACCATCTTCGCCCCTAGCAACGAGGCCTGGGCCTCCTT
GCCAGCTGAAGTGCTGGACTCCCTGGTCAGCAATGTCAACATTGAGCTGC
TCAATGCCCTCCGCTACCATATGGTGGGCAGGCGAGTCCTGACTGATGAG
CTGAAACACGGCATGACCCTCACCTCTATGTACCAGAATTCCAACATCCA
GATCCACCACTATCCTAATGGGATTGTAACTGTGAACTGTGCCCGGCTCCT
GAAAGCCGACCACCATGCAACCAACGGGGTGGTGCACCTCATCGATAAGG
TCATCTCCACCATCACCAACAACATCCAGCAGATCATTGAGATCGAGGAC
ACCTTTGAGACCCTTCGGGCTGCTGTGGCTGCATCAGGGCTCAACACGAT
GCTTGAAGGTAACGGCCAGTACACGCTTTTGGCCCCGACCAATGAGGCCT

TCGAGAAGATCCCTAGTGAGACTTTGAACCGTATCCTGGGCGACCCAGAA
GCCCTGAGAGACCTGCTGAACAACCACATCTTGAAGTCAGCTATGTGTGC
TGAAGCCATCGTTGCGGGGCTGTCTGTAGAGACCCTGGAGGGCACGACAC
TGGAGGTGGGCTGCAGCGGGGACATGCTCACTATCAACGGGAAGGCGATC
ATCTCCAATAAAGACATCCTAGCCACCAACGGGGTGATCCACTACATTGA
TGAGCTACTCATCCCAGACTCAGCCAAGACACTATTTGAATTGGCTGCAG
AGTCTGATGTGTCCACAGCCATTGACCTTTTCAGACAAGCCGGCCTCGGCA
ATCATCTCTCTGGAAGTGAGCGGTTGACCCTCCTGGCTCCCCTGAATTCTG
TATTCAAAGATGGAACCCCTCCAATTGATGCCCATACAAGGAATTTGCTTC
GGAACCACATAATTAAAGACCAGCTGGCCTCTAAGTATCTGTACCATGGA
CAGACCCTGGAAACTCTGGGCGGCAAAAAACTGAGAGTTTTTGTTTATCG
TAATAGCCTCTGCATTGAGAACAGCTGCATCGCGGCCCACGACAAGAGGG
GGAGGTACGGGACCCTGTTCACGATGGACCGGGTGCTGACCCCCCCAATG
GGGACTGTCATGGATGTCCTGAAGGGAGACAATCGCTTTAGCATGCTGGT
AGCTGCCATCCAGTCTGCAGGACTGACGGAGACCCTCAACCGGGAAGGAG
TCTACACAGTCTTTGCTCCCACAAATGAAGCCTTCCGAGCCCTGCCACCAA
GAGAACGGAGCAGACTCTTGGGAGATGCCAAGGAACTTGCCAACATCCTG
AAATACCACATTGGTGATGAAATCCTGGTTAGCGGAGGCATCGGGGCCCT
GGTGCGGCTAAAGTCTCTCCAAGGTGACAAGCTGGAAGTCAGCTTGAAAA
ACAATGTGGTGAGTGTCAACAAGGAGCCTGTTGCCGAGCCTGACATCATG
GCCACAAATGGCGTGGTCCATGTCATCACCAATGTTCTGCAGCCTCCAGCC
AACAGACCTCAGGAAAGAGGGGATGAACTTGCAGACTCTGCGCTTGAGAT
CTTCAAACAAGCATCAGCGTTTTCCAGGGCTTCCCAGAGGTCTGTGCGACT
AGCCCCTGTCTATCAAAAGTTATTAGAGAGGATGAAGCATTAGCTTGAAG
CACTACAGGAGGAATGCACCACGGCAGCTCTCCGCCAATTTCTCTCAGAT
TTCCACAGAGACTGTTTGAATGTTTTCAAAACCAAGTATCACACTTTAATG
TACATGGGCCGCACCATAATGAGATGTGAGCCTTGTGCATGTGGGGGAGG
AGGGAGAGAGATGTACTTTTTAAATCATGTTCCCCCTAAACATGGCTGTTA
ACCCACTGCATGCAGAAACTTGGATGTCACTGCCTGACATTCACTTCCAGA
GAGGACCTATCCCAAATGTGGAATTGACTGCCTATGCCAAGTCCCTGGAA
AAGGAGCTTCAGTATTGTGGGGCTCATAAAACATGAATCAAGCAATCCAG
CCTCATGGGAAGTCCTGGCACAGTTTTTGTAAAGCCCTTGCACAGCTGGA
GAAATGGCATCATTATAAGCTATGAGTTGAAATGTTCTGTCAAATGTGTCT
CACATCTACACGTGGCTTGGAGGCTTTTATGGGGCCCTGTCCAGGTAGAA
AAGAAATGGTATGTAGAGCTTAGATTTCCCTATTGTGACAGAGCCATGGT
GTGTTTGTAATAATAAAACCAAAGAAACATA SEQ ID NO: 80

EGF-Containing Fibulin-Like Extracellular Matrix Protein 2

>gi|8393298|ref|NM_016938.1| Homo sapiens EGF-containing fibulin-like extracellular matrix protein 2 (EFEMP2), mRNA | qPCR assay_on_demand_context match [1248..1272]

CAAGCTTGGCACGAGGGCAGGCATTGCCCGAGCCAGCCGAGCCGC
CAGAGCCGCGGGCCGCGCGGGTGTCGCGGGCCCAACCCCAGGATGCTCCC
CTGCGCCTCCTGCCTACCCGGGTCTCTACTGCTCTGGGCGCTGCTACTGTT
GCTCTTGGGATCAGCTTCTCCTCAGGATTCTGAAGAGCCCGACAGCTACAC
GGAATGCACAGATGGCTATGAGTGGGACCCAGACAGCCAGCACTGCCGG
GATGTCAACGAGTGTCTGACCATCCCTGAGGCCTGCAAGGGGGAAATGAA
GTGCATCAACCACTACGGGGGCTACTTGTGCCTGCCCCGCTCCGCTGCCGT

CATCAACGACCTACACGGCGAGGGACCCCCGCCACCAGTGCCTCCCGCTC
AACACCCCAACCCCTGCCCACCAGGCTATGAGCCCGACGATCAGGACAGC
TGTGTGGATGTGGACGAGTGTGCCCAGGCCCTGCACGACTGTCGCCCCAG
CCAGGACTGCCATAACTTGCCTGGCTCCTATCAGTGCACCTGCCCTGATGG
TTACCGCAAGATCGGGCCCGAGTGTGTGGACATAGACGAGTGCCGCTACC
GCTACTGCCAGCACCGCTGCGTGAACCTGCCTGGCTCCTTCCGCTGCCAGT
GCGAGCCGGGCTTCCAGCTGGGGCCTAACAACCGCTCCTGTGTTGATGTG
AACGAGTGTGACATGGGGGCCCCATGCGAGCAGCGCTGCTTCAACTCCTA
TGGGACCTTCCTGTGTCGCTGCCACCAGGGCTATGAGCTGCATCGGGATG
GCTTCTCCTGCAGTGATATTGATGAGTGTAGCTACTCCAGCTACCTCTGTC
AGTACCGCTGCGTCAACGAGCCAGGCCGTTTCTCCTGCCACTGCCCACAG
GGTTACCAGCTGCTGGCCACACGCCTCTGCCAAGACATTGATGAGTGTGA
GTCTGGTGCGCACCAGTGCTCCGAGGCCCAAACCTGTGTCAACTTCCATG
GGGGCTACCGCTGCGTGGACACCAACCGCTGCGTGGAGCCCTACATCCAG
GTCTCTGAGAACCGCTGTCTCTGCCCGGCCTCCAACCCTCTATGTCGAGAG
CAGCCTTCATCCATTGTGCACCGCTACATGACCATCACCTCGGAGCGGAG
AGTACCCGCTGACGTGTTCCAGATCCAGGCGACCTCCGTCTACCCCGGTGC
CTACAATGCCTTTCAGATCCGTGCTGGAAACTCGCAGGGGGACTTTTACAT
TAGGCAAATCAACAACGTCAGCGCCATGCTGGTCCTCGCCCGGCCGGTGA
CGGGCCCCCGGGAGTACGTGCTGGACCTGGAGATGGTCACCATGAATTCC
CTCATGAGCTACCGGGCCAGCTCTGTACTGAGGCTCACCGTCTTTGTAGGG
GCCTACACCTTCTGAGGAGCAGGAGGGAGCCACCCTCCCTGCAGCTACCC
TAGCTGAGGAGCCTGTTGTGAGGGGCAGAATGAGAAAGGCCCAGGGGCC
CCCATTGACAGGAGCTGGGAGCTCTGCACCACGAGCTTCAGTCACCCCGA
GAGGAGAGGAGGTAACGAGGAGGGCGGACTCCAGGCCCCGGCCCAGAGA
TTTGGACTTGGCTGGCTTGCAGGGGTCCTAAGAAACTCCACTCTGGACAG
CGCCAGGAGGCCCTGGGTTCCATTCCTAACTCTGCCTCAAACTGTACATTT
GGATAAGCCCTAGTAGTTCCCTGGGCCTGTTTTTCTATAAAACGAGGCAAC
TGG SEQ ID NO: 81

Lumican

>gi|21359858|ref|NM_002345.2| Homo sapiens lumican (LUM), mRNA | qPCR forward_primer match [61..84] | qPCR reverse_primer match [182..162] | qPCR probe match [117..152]

GTATCACTCAGAATCTGGCAGCCAGTTCCGTCCTGACAGAGTTCAC
AGCATATATTGGTGGATTCTTGTCCATAGTGCATCTGCTTTAAGAATTAAC
GAAAGCAGTGTCAAGACAGTAAGGATTCAAACCATTTGCCAAAAATGAGT
CTAAGTGCATTTACTCTCTTCCTGGCATTGATTGGTGGTACCAGTGGCCAG
TACTATGATTATGATTTTCCCCTATCAATTTATGGGCAATCATCACCAAAC
TGTGCACCAGAATGTAACTGCCCTGAAAGCTACCCAAGTGCCATGTACTG
TGATGAGCTGAAATTGAAAAGTGTACCAATGGTGCCTCCTGGAATCAAGT
ATCTTTACCTTAGGAATAACCAGATTGACCATATTGATGAAAAGGCCTTTG
AGAATGTAACTGATCTGCAGTGGCTCATTCTAGATCACAACCTTCTAGAA
AACTCCAAGATAAAAGGGAGAGTTTTCTCTAAATTGAAACAACTGAAGAA
GCTGCATATAAACCACAACAACCTGACAGAGTCTGTGGGCCCACTTCCCA
AATCTCTGGAGGATCTGCAGCTTACTCATAACAAGATCACAAAGCTGGGC
TCTTTTGAAGGATTGGTAAACCTGACCTTCATCCATCTCCAGCACAATCGG
CTGAAAGAGGATGCTGTTTCAGCTGCTTTTAAAGGTCTTAAATCACTCGAA

TACCTTGACTTGAGCTTCAATCAGATAGCCAGACTGCCTTCTGGTCTCCCT
GTCTCTCTTCTAACTCTCTACTTAGACAACAATAAGATCAGCAACATCCCT
GATGAGTATTTCAAGCGTTTTAATGCATTGCAGTATCTGCGTTTATCTCAC
AACGAACTGGCTGATAGTGGAATACCTGGAAATTCTTTCAATGTGTCATCC
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AATGAAAACCTTGAAAACTATTACCTGGAGGTCAATCAACTTGAGAAGTT
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TCAAGCATTTGCGTTTGGATGGCAATCGCATCTCAGAAACCAGTCTTCCAC
CGGATATGTATGAATGTCTACGTGTTGCTAACGAAGTCACTCTTAATTAAT
ATCTGTATCCTGGAACAATATTTTATGGTTATGTTTTTCTGTGTGTCAGTTT
TCATAGTATCCATATTTTATTACTGTTTATTACTTCCATGAATTTTAAAATC
TGAGGGAAATGTTTTGTAAACATTTATTTTTTTTAAAGAAAAGATGAAAG
GCAGGCCTATTTCATCACAAGAACACACACATATACACGAATAGACATCA
AACTCAATGCTTTATTTGTAAATTTAGTGTTTTTTTATTTCTACTGTCAAAT
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CCTTAAATCTTAATGTTCCTCAAAGCTTGGATTAAATACATATGGATGTTA
CTCTCTTGCACCAAATTATCTTGATACATTCAAATTTGTCTGGTTAAAAAA
TAGGTGGTAGATATTGAGGCCAAGAATATTGCAAAATACATGAAGCTTCA
TGCACTTAAAGAAGTATTTTTAGAATAAGAATTTGCATACTTACCTAGTGA
AACTTTTCTAGAATTATTTTTCACTCTAAGTCATGTATGTTTCTCTTTGATT
ATTTGCATGTTATGTTTAATAAGCTACTAGCAAAATAAAACATAGCAAAT
GAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA SEQ ID NO: 82

Stannin

>gi|29893560|ref|NM_003498.3| Homo sapiens stannin (SNN), mRNA

AGCGGGGCCGGACCGGGCGGGCGGAGCCGGGCCCGCGGGGCTGCT
GCGGGGCGATCGGGCCGGGCCGCTGCCGCGCCATGGACTCCCGTGTCCAG
CCTGAGTTCCAGCCTCACTGAGTGGCCACCCCCAAAGTGCTGCCAGCCGA
GGAAGCCCCCAGCACTGACCATGTCTATTATGGACCACAGCCCCACCACG
GGCGTGGTCACAGTCATCGTCATCCTCATTGCCATCGCGGCCCTGGGGGCC
TTGATCCTGGGCTGCTGGTGCTACCTGCGGCTGCAGCGCATCAGCCAGTCA
GAGGACGAGGAGAGCATCGTGGGGGATGGGGAGACCAAGGAACCCTTCC
TGCTGGTGCAGTATTCGGCCAAGGGACCGTGCGTGGAGAGAAAGGCCAA
GCTGATGACTCCCAACGGCCCGGAAGTCCACGGCTGAGCCAGGATGCAAG
GCTCCTGGTCCTGTTTGCAGCCGGCCAAGAGGCGCTGGGAGGGGCAAAAC
CATACGGATGCGCTGCTGTCTGAGAGGAAGGGCTGACACTTGCTGGCATG
GCCTCTGCGGGCTTCGTCATCGCATGCACTGATGCCCGGGGACCTGGCTGT
CCTGGGCTTCCCCTCGGCCTCCAGGTGAGGCTGCCCATTGCAGGCACTGG
GCAGGCCTGACCTTGCTGGGGCTCATGGCCCTGTAGCGCTTTTGTTACTTG
AATGTCTAGCTGAGCCTGTTTTTGATGGAGCTACTACTGTAATGCGTGAAC
TAACAAACCTGTGAACTGTAAATAGGCCCCTGGAAGCACGTGCTTAAGCC
CTTTTGCTGATTTTTAAAAATATCATCTAGCGCACACGGGACTGGTATTCT
GGCTGTACTAATGACAAGCTGAGTCAAGACCCTGGAGGGTCATAGGCTTG
TAAAGGCCCACGCCACACTCGGCAGGGGTCTCTCATGTGTGTCCATCTGC
GTGTATGTCAAGGAAGTGAGATGCCAATTTGGGGTCTTGAGGCTGACCAG
TTGGGGTGCTTGGGTGATCTCTGCTTCATTAGTCATGGGTGGAAGAAAAA
CCACACCCCCCGCACCCCTCCGTTCTTTCTGCATAGACTCACTTGTTAAAT
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TGGGAGCCAGTGAAGGGCGAGTCAGGGCACGCGTCCTGGAGGCTGCCAG

CGTCCTTGTAGCAGAGCAGTTTCTTGCCGCTTGGGTCTTCAGCACGCCAAG
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TCCTCATAGAGCCTGTGGTGGCTACTTTTGGTCTGAAACCCACTTGGCCCA
GGAAAGAGAAAAGGTTGTATGTTTTGTGTTGGTGTTTCCTATTTTCTGCAC
TGGAGGGGAGGGGACTGTTGAGGTTCTGTCTTTTTTCTTCTTTTCCTCTTCC
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GTTCTGACTTCACTTTCCTCAGCGGGTCTCCAGTCCCCTGACCCAGCTCTA
AAGGCACTTAGGACCCAGGGAACATTTCTCACGTGCACATTCCCCTAAGA
GCCACCAGACTGCTTCCTGCCAGCCTGTGCTTGCGGCAGGGAGCCGGGGC
AGGGCAGAGGTGAACTTGAAGTTCAGGACTTGACTCTCCCACAGGTGGTG
AGCTGGTGGCTCTCTGGTGAGCTAGTGTCTCCACAGCCTGTCTCCAAGGCC
TCCCCTATGTACATTTCAGTGAGCTCACTTTGATTTTTAATCCCACCACAA
GCACATACTAATTTTATTTATGATTCAAATGTGACTCGTGCCTGCCCATCC
CTGTAATAGATGGAAGGTCAGCCCCGGCTTAACCACAGAGCACTGGCCCT
TCATGGCTGAGCTCAGAGCTCTGGCCTCCTGCTCAGACTAAAGGCACCTCC
TCTGGCCTCACCCAAGCCTCTTCTAAAAACCATGTTGAATGAATCCACGTT
CTGGAACCCCGAGGCGGGAGAAGTAGGGAGCTGTTCGTTTAAGCAGCATA
CACCTAAATTGGGGGTTTAAACATTAAGTAGGAGCTTGGGGTGGAAGAGG
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CAGCAGAAGCCCTGCAGGCTGGGTGGGCAGGACACGTGGTGGGGGCCAC
TGAAACCAGGCCTAGGAGGGAGAACAAGTTCCAAAGGTGCCGACTGGAA
GAAGGGGGTAAAAGTTTGCTTTGGTGAGTGAGAAAAGGCTGGGGCGTGTG
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ACGCAGCAACAGCTAGGATCTGCATTTTCAGGTTCCGAGCCTGACCCCTG
GAACTGACCAGCGCTCGATTGTCAGCCTTGGCCTGGGGTTTTGACCTTGCC
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CTTCTGGAGGTTTTCTTATCCTACTCCCTGGTGCCAGGGACGTACCTGGGA
GTTTGAATCAGGCCCATTTGAGCGTGGCAGCCGTGTTGGGTGAAGGTCCG
GGGCTCGGTGAGGCACTGGGGGGGTTTTCGGGAGGAAAATGAAAATGCTT
CTAGAATGAGTGAACCACATCATAGCTCTCACTGTTTTTTCAATAGCTACT
TTTTTTAGCAGACACCAGAGCCACACTCAAATGGCTAAGTAGGTTATGAC
CTCTCTGGATTATTTTTGAATGCCCAACTGTTGCATTCAAGTTTTCTGACTA
ATAAGAAATTAAGCATTCATCCTTCGTATCACTGCAGAAGCAACAGTGGG
GGCACAGGGAGGGAACTCTTGACACTGAGCCACTAAAATATGGACTAATT
TTTTGGACAAATCTTCAAACGGACTGTGCTACTGTATTTGTCTCAAAGCTA
CCAAGTTTGTGCAATAAGTGGAAGGGATGTCATCCTTCTTCAATAAATGCT
GAATGACATTCAAGCTGATTTTCTAGACCACTGAGAAAATCTTTATTTACA
ATAAATTTCAATAAAATTTGCATAAATATATTCCCAAAAAAAAAAAAAAAA
AAAAAAGAAAAAAAAAAAAA SEQ ID NO: 83

Secreted Phosphoprotein 1

>gi|38146097|ref|NM_000582.2| Homo sapiens secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) (SPP1), mRNA | qPCR assay_on_demand_context match [253..277]

CTCCCTGTGTTGGTGGAGGATGTCTGCAGCAGCATTTAAATTCTGG
GAGGGCTTGGTTGTCAGCAGCAGCAGGAGGAGGCAGAGCACAGCATCGT
CGGGACCAGACTCGTCTCAGGCCAGTTGCAGCCTTCTCAGCCAAACGCCG
ACCAAGGAAAACTCACTACCATGAGAATTGCAGTGATTTGCTTTTGCCTCC
TAGGCATCACCTGTGCCATACCAGTTAAACAGGCTGATTCTGGAAGTTCTG
AGGAAAAGCAGCTTTACAACAAATACCCAGATGCTGTGGCCACATGGCTA
AACCCTGACCCATCTCAGAAGCAGAATCTCCTAGCCCCACAGACCCTTCC
AAGTAAGTCCAACGAAAGCCATGACCACATGGATGATATGGATGATGAA
GATGATGATGACCATGTGGACAGCCAGGACTCCATTGACTCGAACGACTC
TGATGATGTAGATGACACTGATGATTCTCACCAGTCTGATGAGTCTCACCA
TTCTGATGAATCTGATGAACTGGTCACTGATTTTCCCACGGACCTGCCAGC
AACCGAAGTTTTCACTCCAGTTGTCCCCACAGTAGACACATATGATGGCC
GAGGTGATAGTGTGGTTTATGGACTGAGGTCAAAATCTAAGAAGTTTCGC
AGACCTGACATCCAGTACCCTGATGCTACAGACGAGGACATCACCTCACA
CATGGAAAGCGAGGAGTTGAATGGTGCATACAAGGCCATCCCCGTTGCCC
AGGACCTGAACGCGCCTTCTGATTGGGACAGCCGTGGGAAGGACAGTTAT
GAAACGAGTCAGCTGGATGACCAGAGTGCTGAAACCCACAGCCACAAGC
AGTCCAGATTATATAAGCGGAAAGCCAATGATGAGAGCAATGAGCATTCC
GATGTGATTGATAGTCAGGAACTTTCCAAAGTCAGCCGTGAATTCCACAG
CCATGAATTTCACAGCCATGAAGATATGCTGGTTGTAGACCCCAAAAGTA
AGGAAGAAGATAAACACCTGAAATTTCGTATTTCTCATGAATTAGATAGT
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CATTTAGTCAAAAGAAAAAATGCTTTATAGCAAAATGAAAGAGAACATGA
AATGCTTCTTTCTCAGTTTATTGGTTGAATGTGTATCTATTTGAGTCTGGAA
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TGCAAACTATCACTGTATTTTAATATTTGTTATTCTCTCATGAATAGAAATT
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GATTATCTTTTTGTGGTGTGAATAAATCTTTTATCTTGAATGTAATAAGAA
TTTGGTGGTGTCAATTGCTTATTTGTTTTCCCACGGTTGTCCAGCAATTAAT
AAAACATAACCTTTTTTACTGCCTAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAA SEQ ID NO: 84

Chondroitin Sulfate Proteoglycan 2

>gi|21361115|ref|NM_004385.2| Homo sapiens chondroitin sulfate proteoglycan 2 (versican) (CSPG2), mRNA | qPCR forward_primer match [10087..10106] | qPCR reverse_primer match [10185..10163] | qPCR probe match [10139..10161]

GCTGCCCCGAGCCTTTCTGGGGAAGAACTCCAGGCGTGCGGACGCA
ACAGCCGAGAACATTAGGTGTTGTGGACAGGAGCTGGGACCAAGATCTTC
GGCCAGCCCCGCATCCTCCCGCATCTTCCAGCACCGTCCCGCACCCTCCGC
ATCCTTCCCCGGGCCACCACGCTTCCTATGTGACCCGCCTGGGCAACGCCG
AACCCAGTCGCGCAGCGCTGCAGTGAATTTTCCCCCCAAACTGCAATAAG
CCGCCTTCCAAGGCCAAGATGTTCATAAATATAAAGAGCATCTTATGGAT
GTGTTCAACCTTAATAGTAACCCATGCGCTACATAAAGTCAAAGTGGGAA
AAAGCCCACCGGTGAGGGGCTCCCTCTCTGGAAAAGTCAGCCTACCTTGT

CATTTTTCAACGATGCCTACTTTGCCACCCAGTTACAACACCAGTGAATTT
CTCCGCATCAAATGGTCTAAGATTGAAGTGGACAAAAATGGAAAAGATTT
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AGGACTACAAAGGGAGAGTGTCTGTGCCCACACATCCCGAGGCTGTGGGC
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CACTGAATTTTGAGGCTGCTCAGAAGGCTTGTTTGGACGTTGGGGCAGTC
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GACTTATGGATTCCGTTCTCCCCAGGAAACTTACGATGTGTATTGTTATGT
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CTTCGAGGAGGCTGCAAAAGAGTGTGAAAACCAGGATGCCAGGCTGGCA
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AGGGCCCAGTGTGGAGGTGGTCTACTTGGGGTGAGAACCCTGTATCGTTT
TGAGAACCAGACAGGCTTCCCTCCCCCTGATAGCAGATTTGATGCCTACTG
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CTGCATCACCCAGTTTATCCAAAGAACCACAAATGGTTTCTGATAGAACT
ACACCAATCATCCCTTTAGTTGATGAATTACCTGTCATTCCAACAGAGTTC
CCTCCCGTGGGAAATATTGTCAGTTTTGAACAGAAAGCCACAGTCCAACC
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CCTCTTGGAAAGCTAGACATATCAGAAATTAAGGAAGAAGTGCTCCAGAG
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CCACTGTATCAAAATGGTCATGGGATGAAGATAATACAACATCCAAGCCT
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CTCATTACTTTAGACACTAGCAGGATAATCACAGAAAGCTTTTTTGAGGTT
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ACTGTTGAGGAAAAGAAAAGGAAGGAGGAGGAGGGAACTACAGGTACGG
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TACCCTTTGAATTAGAAAGTCCAAATGTAGCTACATCTAGTGATTCAGGTA
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AGGGAAATAGTGATTTCAGAGCGATTAGGAGAACCAAATTATGGGGCAG
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GTAATGATGGAAGGCTCTGGAGATGCAGCATTTAGGGACACCCAGACTTC
ACCATCTACAGTACCTACTTCAGTTCACATCAGTCACATATCTGACTCAGA
AGGACCCAGTAGCACCATGGTCAGCACTTCAGCCTTCCCCTGGGAAGAGT
TTACATCCTCAGCTGAGGGCTCAGGTGAGCAACTGGTCACAGTCAGCAGC
TCTGTTGTTCCAGTGCTTCCCAGTGCTGTGCAAAAGTTTTCTGGTACAGCT
TCCTCCATTATCGACGAAGGATTGGGAGAAGTGGGTACTGTCAATGAAAT
TGATAGAAGATCCACCATTTTACCAACAGCAGAAGTGGAAGGTACGAAAG
CTCCAGTAGAGAAGGAGGAAGTAAAGGTCAGTGGCACAGTTTCAACAAA
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AAACAATCTTTGATTCACAGACATTTACTGAAACTGAACTCAAAACCACA
GATTATTCTGTACTAACAACAAAGAAAACTTACAGTGATGATAAAGAAAT
GAAGGAGGAAGACACTTCTTTAGTTAACATGTCTACTCCAGATCCAGATG
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GAAAGGCATGAGACCAACAATTCAAGAGTCAGATACTGAGCTCTTATTCT
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GTGAATTTTACTGAAGTGGAACAAATCAATAACACATTATATCCCCACAC
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ATATTGACAATTACAGAGAGTACCATCCTTGAAATTCTACCTGAGCTGAC
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GATCTATGAGGCAGCTGTCAACCTTTCTTTAACTGAGGAAACATTTGAGG
GCTCTGCTGATGTTCTGGCTAGCTACACTCAGGCAACACATGATGAATCA
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CAAGCACTTTGTCTGATGGTCAAGCTATTGCAGACCAAAGTGAAATAATA
CCAACATTGGGCCAATTTGAAAGGACTCAGGAGGAGTATGAAGACAAAA
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CACACAGAGATCCCCCAGCCCAGTGCTCTGCCAGGAATAGACGTCGGCTC
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GAACTGTTATTACAACTGCCGATGAAATTGAATTAGAAGGTGCTACACAG
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GTGCCTTGGCTAAGTCCACAGACTTCTGAGAGGCCCACGCTTTCTTCTTCT
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GGTGCACTTTGTGAGCAAGATACCGAGACATGTGACTATGGCTGGCACAA
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CAGCACACTGCAATACGAGAATTGGAGACCCAACCAGCCAGACAGCTTCT
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AGTCGAGGCGCTGATCCCTAAAATGGCGAACATGTGTTTTCATCATTTCAG
CCAAAGTCCTAACTTCCTGTGCCTTTCCTATCACCTCGAGAAGTAATTATC
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CCCAAAACATTTTAAATGAAAGTATTGGCATTCAAAAAGACAGCAGACAA
AATGAAAGAAAATGAGAGCAGAAAGTAAGCATTTCCAGCCTATCTAATTT
CTTTAGTTTTCTATTTGCCTCCAGTGCAGTCCATTTCCTAATGTATACCAGC
CTACTGTACTATTTAAAATGCTCAATTTCAGCACCGATGGCCATGTAAATA
AGATGATTTAATGTTGATTTTAATCCTGTATATAAAATAAAAAGTCACAAT
GAGTTTGGGCATATTTAATGATGATTATGGAGCCTTAGAGGTCTTTAATCA
TTGGTTCGGCTGCTTTTATGTAGTTTAGGCTGGAAATGGTTTCACTTGCTCT
TTGACTGTCAGCAAGACTGAAGATGGCTTTTCCTGGACAGCTAGAAAACA
CAAAATCTTGTAGGTCATTGCACCTATCTCAGCCATAGGTGCAGTTTGCTT
CTACATGATGCTAAAGGCTGCGAATGGGATCCTGATGGAACTAAGGACTC
CAATGTCGAACTCTTCTTTGCTGCATTCCTTTTTCTTCACTTACAAGAAAGG
CCTGAATGGAGGACTTTTCTGTAACCAGG SEQ ID NO: 85

N-Acylsphingosine Amidohydrosase 1

>gi|30089929|ref|NM_004315.2| Homo sapiens N-acylsphingosine amidohydrolase (acid ceramidase) 1 (ASAH1), transcript variant 2, mRNA | qPCR forward_primer match [1212..1228] | qPCR reverse_primer match [1290..1266] | qPCR probe match [1233..1260]

GGACTTTGAAATCCAACCCGGTCACCTACCCGCGCGACTGTGTCCA
CGGATGGCACGAAAGCCAAGCGAGTCCCCCTGCCGAGCTACTCGCGTCCG
CCTCCTCCCAAGCTGAGCTCTGCTCCGCCCACCTGAGTCCTTCGCCAGTTA
GGAGGAAACACAGCCGCTTAATGAACTGCTGCATCGGGCTGGGAGAGAA
AGCTCGCGGGTCCCACCGGGCCTCCTACCCAAGTCTCAGCGCGCTTTTCAC
CGAGGCCTCAATTCTGGGATTTGGCAGCTTTGCTGTGAAAGCCCAATGGA
CAGAGGACTGCAGAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGA
GGTGCAGTTCCATGGTACACCATAAATCTTGACTTACCACCCTACAAAAG
ATGGCATGAATTGATGCTTGACAAGGCACCAATGCTAAAGGTTATAGTGA
ATTCTCTGAAGAATATGATAAATACATTCGTGCCAAGTGGAAAAGTTATG
CAGGTGGTGGATGAAAAATTGCCTGGCCTACTTGGCAACTTTCCTGGCCCT
TTTGAAGAGGAAATGAAGGGTATTGCCGCTGTTACTGATATACCTTTAGG
AGAGATTATTTCATTCAATATTTTTTATGAATTATTTACCATTTGTACTTCA
ATAGTAGCAGAAGACAAAAAAAGGTCATCTAATACATGGGAGAAACATGG
ATTTTGGAGTATTTCTTGGGTGGAACATAAATAATGATACCTGGGTCATAA
CTGAGCAACTAAAACCTTTAACAGTGAATTTGGATTTCCAAAGAAACAAC
AAAACTGTCTTCAAGGCTTCAAGCTTTGCTGGCTATGTGGGCATGTTAACA
GGATTCAAACCAGGACTGTTCAGTCTTACACTGAATGAACGTTTCAGTATA
AATGGTGGTTATCTGGGTATTCTAGAATGGATTCTGGGAAAGAAAGATGC
CATGTGGATAGGGTTCCTCACTAGAACAGTTCTGGAAAATAGCACAAGTT
ATGAAGAAGCCAAGAATTTATTGACCAAGACCAAGATATTGGCCCCAGCC
TACTTTATCCTGGGAGGCAACCAGTCTGGGGAAGGTTGTGTGATTACACG
AGACAGAAAGGAATCATTGGATGTATATGAACTCGATGCTAAGCAGGGTA
GATGGTATGTGGTACAAACAAATTATGACCGTTGGAAACATCCCTTCTTCC
TTGATGATCGCAGAACGCCTGCAAAGATGTGTCTGAACCGCACCAGCCAA
GAGAATATCTCATTTGAAACCATGTATGATGTCCTGTCAACAAAACCTGTC

CTCAACAAGCTGACCGTATACACAACCTTGATAGATGTTACCAAAGGTCA
ATTCGAAACTTACCTGCGGGACTGCCCTGACCCTTGTATAGGTTGGTGAGC
ACACGTCTGGCCTACAGAATGCGGCCTCTGAGACATGAAGACACCATCTC
CATGTGACCGAACACTGCAGCTGTCTGACCTTCCAAAGACTAAGACTCGC
GGCAGGTTCTCTTTGAGTCAAAAGCTTGTCTTCGTCCATCTGTTGACAAAT
GACAGACCTTTTTTTTTCCCCCATCAGTTGATTTTTCTTATTTACAGATAAC
TTCTTTAGGGGAAGTAAAACAGTCATCTAGAATTCACTGAGTTTTGTTTCA
CTTTGACATTTGGGGATCTGGTGGGCAGTCGAACCATGGTGAACTCCACCT
CCGTGGAATAAATGGAGATTCAGCGTGGGTGTTGAATCCAGCACGTCTGT
GTGAGTAACGGGACAGTAAACACTCCACATTCTTCAGTTTTTCACTTCTAC
CTACATATTTGTATGTTTTTCTGTATAACAGCCTTTTCCTTCTGGTTCTAAC
TGCTGTTAAAATTAATATATCATTATCTTTGCTGTTATTGACAGCGATATA
ATTTTATTACATATGATTAGAGGGATGAGACAGACATTCACCTGTATATTT
CTTTTAATGGGCACAAAATGGGCCCTTGCCTCTAAATAGCACTTTTTGGGG
TTCAAGAAGTAATCAGTATGCAAAGCAATCTTTTATACAATAATTGAAGT
GTTCCCTTTTTCATAATTACTGTACTTCCCAGTAACCCTAAGGAAGTTGCT
AACTTAAAAAACTGCATCCCACGTTCTGTTAATTTAGTAAATAAACAAGTC
AAAGACTTGTGGAAAATAGGAAGTGAACCCATATTTTAAATTCTCATAAG
TAGCATTCATGTAATAAACAGGTTTTTAGTTTGTTCTTCAGATTGATAGGG
AGTTTTAAAGAAATTTTAGTAGTTACTAAAATTATGTTACTGTATTTTTCA
GAAATCAAACTGCTTATGAAAAGTACTAATAGAACTTGTTAACCTTTCTAA
CCTTCACGATTAACTGTGAAATGTACGTCATTTGTGCAAGACCGTTTGTCC
ACTTCATTTTGTATAATCACAGTTGTGTTCCTGACACTCAATAAACAGTCA
TTGGAAAGAGTGCCAGTCAGCAGTCATGCA SEQ ID NO: 86

N-Acylsphingosine Amidohydrolase 1 Transcript Variant 1

>gi|30089927|ref|NM_177924.1| Homo sapiens N-acylsphingosine amidohydrolase (acid ceramidase) 1 (ASAH1), transcript variant 1, mRNA | qPCR forward_primer match [1050..1066] | qPCR reverse_primer match [1128..1104] | qPCR probe match [1071..1098]

GGCTCTTCTTTGCCTCTGCTGGAGTCCGGGGAGTGGCGTTGGCTGCT
AGAGCGATGCCGGGCCGGAGTTGCGTCGCCTTAGTCCTCCTGGCTGCCGC
CGTCAGCTGTGCCGTCGCGCAGCACGCGCCGCCGTGGACAGAGGACTGCA
GAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGAGGTGCAGTTCCA
TGGTACACCATAAATCTTGACTTACCACCCTACAAAAGATGGCATGAATT
GATGCTTGACAAGGCACCAATGCTAAAGGTTATAGTGAATTCTCTGAAGA
ATATGATAAATACATTCGTGCCAAGTGGAAAAGTTATGCAGGTGGTGGAT
GAAAAATTGCCTGGCCTACTTGGCAACTTTCCTGGCCCTTTTGAAGAGGAA
ATGAAGGGTATTGCCGCTGTTACTGATATACCTTTAGGAGAGATTATTTCA
TTCAATATTTTTTATGAATTATTTACCATTTGTACTTCAATAGTAGCAGAA
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AACCTTTAACAGTGAATTTGGATTTCCAAAGAAACAACAAAACTGTCTTC
AAGGCTTCAAGCTTTGCTGGCTATGTGGGCATGTTAACAGGATTCAAACC
AGGACTGTTCAGTCTTACACTGAATGAACGTTTCAGTATAAATGGTGGTTA
TCTGGGTATTCTAGAATGGATTCTGGGAAAGAAAGATGCCATGTGGATAG
GGTTCCTCACTAGAACAGTTCTGGAAAATAGCACAAGTTATGAAGAAGCC

AAGAATTTATTGACCAAGACCAAGATATTGGCCCCAGCCTACTTTATCCTG
GGAGGCAACCAGTCTGGGGAAGGTTGTGTGATTACACGAGACAGAAAGG
AATCATTGGATGTATATGAACTCGATGCTAAGCAGGGTAGATGGTATGTG
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AGAACGCCTGCAAAGATGTGTCTGAACCGCACCAGCCAAGAGAATATCTC
ATTTGAAACCATGTATGATGTCCTGTCAACAAAACCTGTCCTCAACAAGCT
GACCGTATACACAACCTTGATAGATGTTACCAAAGGTCAATTCGAAACTT
ACCTGCGGGACTGCCCTGACCCTTGTATAGGTTGGTGAGCACACGTCTGG
CCTACAGAATGCGGCCTCTGAGACATGAAGACACCATCTCCATGTGACCG
AACACTGCAGCTGTCTGACCTTCCAAAGACTAAGACTCGCGGCAGGTTCT
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GAAGTAAAACAGTCATCTAGAATTCACTGAGTTTTGTTTCACTTTGACATT
TGGGGATCTGGTGGGCAGTCGAACCATGGTGAACTCCACCTCCGTGGAAT
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GGGACAGTAAACACTCCACATTCTTCAGTTTTTCACTTCTACCTACATATT
TGTATGTTTTTCTGTATAACAGCCTTTTCCTTCTGGTTCTAACTGCTGTTAA
AATTAATATATCATTATCTTTGCTGTTATTGACAGCGATATAATTTTATTAC
ATATGATTAGAGGGATGAGACAGACATTCACCTGTATATTTCTTTTAATGG
GCACAAAATGGGCCCTTGCCTCTAAATAGCACTTTTTGGGGTTCAAGAAG
TAATCAGTATGCAAAGCAATCTTTTATACAATAATTGAAGTGTTCCCTTTT
TCATAATTACTGTACTTCCCAGTAACCCTAAGGAAGTTGCTAACTTAAAAA
ACTGCATCCCACGTTCTGTTAATTTAGTAAATAAACAAGTCAAAGACTTGT
GGAAAATAGGAAGTGAACCCATATTTTAAATTCTCATAAGTAGCATTCAT
GTAATAAACAGGTTTTTAGTTTGTTCTTCAGATTGATAGGGAGTTTTAAAG
AAATTTTAGTAGTTACTAAAATTATGTTACTGTATTTTTCAGAAATCAAAC
TGCTTATGAAAAGTACTAATAGAACTTGTTAACCTTTCTAACCTTCACGAT
TAACTGTGAAATGTACGTCATTTGTGCAAGACCGTTTGTCCACTTCATTTT
GTATAATCACAGTTGTGTTCCTGACACTCAATAAACAGTCATTGGAAAGA
GTGCCAGTCAGCAGTCATGCA SEQ ID NO: 87

Protease, Serine 11

>gi|21327712|ref|NM_002775.2| Homo sapiens protease, serine, 11 (IGF binding) (PRSS11), mRNA | qPCR forward_primer match [1030..1048] | qPCR reverse_primer match [1106..1083] | qPCR probe match [1080..1050]

CCGGCCCTCGCCCTGTCCGCCGCCACCGCCGCCGCCGCCAGAGTCG
CCATGCAGATCCCGCGCGCCGCTCTTCTCCCGCTGCTGCTGCTGCTGCTGG
CGGCGCCCGCCTCGGCGCAGCTGTCCCGGGCCGGCCGCTCGGCGCCTTTG
GCCGCCGGGTGCCCAGACCGCTGCGAGCCGGCGCGCTGCCCGCCGCAGCC
GGAGCACTGCGAGGGCGGCCGGGCCCGGGACGCGTGCGGCTGCTGCGAG
GTGTGCGGCGCGCCCGAGGGCGCCGCGTGCGGCCTGCAGGAGGGCCCGTG
CGGCGAGGGGCTGCAGTGCGTGGTGCCCTTCGGGGTGCCAGCCTCGGCCA
CGGTGCGGCGGCGCGCGCAGGCCGGCCTCTGTGTGTGCGCCAGCAGCGAG
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CGCCGCCAGCCGCCGCTCCGAGAGGCTGCACCGGCCGCCGGTCATCGTCC
TGCAGCGCGGAGCCTGCGGCCAAGGGCAGGAAGATCCCAACAGTTTGCGC
CATAAATATAACTTTATCGCGGACGTGGTGGAGAAGATCGCCCCTGCCGT
GGTTCATATCGAATTGTTTCGCAAGCTTCCGTTTTCTAAACGAGAGGTGCC

GGTGGCTAGTGGGTCTGGGTTTATTGTGTCGGAAGATGGACTGATCGTGA
CAAATGCCCACGTGGTGACCAACAAGCACCGGGTCAAAGTTGAGCTGAAG
AACGGTGCCACTTACGAAGCCAAAATCAAGGATGTGGATGAGAAAGCAG
ACATCGCACTCATCAAAATTGACCACCAGGGCAAGCTGCCTGTCCTGCTG
CTTGGCCGCTCCTCAGAGCTGCGGCCGGGAGAGTTCGTGGTCGCCATCGG
AAGCCCGTTTTCCCTTCAAAACACAGTCACCACCGGGATCGTGAGCACCA
CCCAGCGAGGCGGCAAAGAGCTGGGGCTCCGCAACTCAGACATGGACTA
CATCCAGACCGACGCCATCATCAACTATGGAAACTCGGGAGGCCCGTTAG
TAAACCTGGACGGTGAAGTGATTGGAATTAACACTTTGAAAGTGACAGCT
GGAATCTCCTTTGCAATCCCATCTGATAAGATTAAAAAGTTCCTCACGGAG
TCCCATGACCGACAGGCCAAAGGAAAAGCCATCACCAAGAAGAAGTATA
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AGTAATTCCTGATACCCCAGCAGAAGCTGGTGGTCTCAAGGAAAACGACG
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ATGAAGATATCATGATCACAGTGATTCCCGAAGAAATTGACCCATAGGCA
GAGGCATGAGCTGGACTTCATGTTTCCCTCAAAGACTCTCCCGTGGATGAC
GGATGAGGACTCTGGGCTGCTGGAATAGGACACTCAAGACTTTTGACTGC
CATTTTGTTTGTTCAGTGGAGACTCCCTGGCCAACAGAATCCTTCTTGATA
GTTTGCAGGCAAAACAAATGTAATGTTGCAGATCCGCAGGCAGAAGCTCT
GCCCTTCTGTATCCTATGTATGCAGTGTGCTTTTTCTTGCCAGCTTGGGCCA
TTCTTGCTTAGACAGTCAGCATTTGTCTCCTCCTTTAACTGAGTCATCATCT
TAGTCCAACTAATGCAGTCGATACAATGCGTAGATAGAAGAAGCCCCACG
GGAGCCAGGATGGGACTGGTCGTGTTTGTGCTTTTCTCCAAGTCAGCACCC
AAAGGTCAATGCACAGAGACCCCGGGTGGGTGAGCGCTGGCTTCTCAAAC
GGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAATTGGGAGCACGATGAC
TCTGAGTTTGAGCTATTAAAGTACTTCTTACACATTG SEQ ID NO: 88

Secreted Frizzled-Related Protein 2

>gi|42656988|ref|XM_050625.4| Homo sapiens secreted frizzled-related protein 2 (SFRP2), mRNA | qPCR forward_primer match [686..703] | qPCR reverse_primer match [750..728] | qPCR probe match [705..726]

CCGGGTCGGAGCCCCCCGGAGCTGCGCGCGGGCTTGCAGCGCCTCG
CCCGCGCTGTCCTCCCGGTGTCCCGCTTCTCCGCGCCCCAGCCGCCGGCTG
CCAGCTTTTCGGGGCCCCGAGTCGCACCCAGCGAAGAGAGCGGGCCCGGG
ACAAGCTCGAACTCCGGCCGCCTCGCCCTTCCCCGGCTCCGCTCCCTCTGC
CCCCTCGGGGTCGCGCGCCCACGATGCTGCAGGGCCCTGGCTCGCTGCTG
CTGCTCTTCCTCGCCTCGCACTGCTGCCTGGGCTCGGCGCGCGGGCTCTTC
CTCTTTGGCCAGCCCGACTTCTCCTACAAGCGCAGCAATTGCAAGCCCATC
CCTGCCAACCTGCAGCTGTGCCACGGCATCGAATACCAGAACATGCGGCT
GCCCAACCTGCTGGGCCACGAGACCATGAAGGAGGTGCTGGAGCAGGCC
GGCGCTTGGATCCCGCTGGTCATGAAGCAGTGCCACCCGGACACCAAGAA
GTTCCTGTGCTCGCTCTTCGCCCCCGTCTGCCTCGATGACCTAGACGAGAC
CATCCAGCCATGCCACTCGCTCTGCGTGCAGGTGAAGGACCGCTGCGCCC
CGGTCATGTCCGCCTTCGGCTTCCCCTGGCCCGACATGCTTGAGTGCGACC
GTTTCCCCCAGGACAACGACCTTTGCATCCCCCTCGCTAGCAGCGACCACC
TCCTGCCAGCCACCGAGGAAGCTCCAAAGGTATGTGAAGCCTGCAAAAAT

AAAAATGATGATGACAACGACATAATGGAAACGCTTTGTAAAAATGATTT
TGCACTGAAAATAAAAGTGAAGGAGATAACCTACATCAACCGAGATACC
AAAATCATCCTGGAGACCAAGAGCAAGACCATTTACAAGCTGAACGGTGT
GTCCGAAAGGGACCTGAAGAAATCGGTGCTGTGGCTCAAAGACAGCTTGC
AGTGCACCTGTGAGGAGATGAACGACATCAACGCGCCCTATCTGGTCATG
GGACAGAAACAGGGTGGGGAGCTGGTGATCACCTCGGTGAAGCGGTGGC
AGAAGGGGCAGAGAGAGTTCAAGCGCATCTCCCGCAGCATCCGCAAGCT
GCAGTGCTAGTCCCGGCATCCTGATGGCTCCGACAGGCCTGCTCCAGAGC
ACGGCTGACCATTTCTGCTCCGGGATCTCAGCTCCCGTTCCCCAAGCACAC
TCCTAGCTGCTCCAGTCTCAGCCTGGGCAGCTTCCCCCTGCCTTTTGCACG
TTTGCATCCCCAGCATTTCCTGAGTTATAAGGCCACAGGAGTGGATAGCTG
TTTTCACCTAAAGGAAAAGCCCACCCGAATCTTGTAGAAATATTCAAACT
AATAAAATCATGAATATTTTTATGAAGTTTAAAAA SEQ ID NO: 89

Phospholipase A2, Group XIIB

>gi|45505134|ref|NM_032562.2| Homo sapiens phospholipase A2, group XIIB (PLA2G12B), mRNA

TGTCCCTGGAATTCTGGGACACTGGCTGGGGTTTGAGGAGAGAAGC
CAGTACCTACCTGGCTGCAGGATGAAGCTGGCCAGTGGCTTCTTGGTTTTG
TGGCTCAGCCTTGGGGGTGGCCTGGCTCAGAGCGACACGAGCCCTGACAC
GGAGGAGTCCTATTCAGACTGGGGCCTTCGGCACCTCCGGGGAAGCTTTG
AATCCGTCAATAGCTACTTCGATTCTTTTCTGGAGCTGCTGGGAGGGAAGA
ATGGAGTCTGTCAGTACAGGTGCCGATATGGAAAGGCACCAATGCCCAGA
CCTGGCTACAAGCCCCAAGAGCCCAATGGCTGCGGCTCCTATTTCCTGGGT
CTCAAGGTACCAGAAAGTATGGACTTGGGCATTCCAGCAATGACAAAGTG
CTGCAACCAGCTGGATGTCTGTTATGACACTTGCGGTGCCAACAAATATC
GCTGTGATGCAAAATTCCGATGGTGTCTCCACTCGATCTGCTCTGACCTTA
AGCGGAGTCTGGGCTTTGTCTCCAAAGTGGAAGCAGCCTGTGATTCCCTG
GTTGACACTGTGTTCAACACCGTGTGGACCTTGGGCTGCCGCCCCTTTATG
AATAGTCAGCGGGCAGCTTGCATCTGTGCAGAGGAGGAGAAGGAAGAGT
TATGAGGAAGAAGTGATTCCTTCCTGGTTTTGAGTGACACCACAGCTGTCA
GCCTTCAAGATGTCAAGTCTTCGAGTCAGCGTGACTCATTCATTCTTCCAA
CAGTTTGGACACCACAAAGCAGGAGAAAGGGAACATTTTTCTACAGCTGG
AAAGTGAGTCCTATCCTTTGAGGAAATTTGAAAAAAGACATGGAGTGGTT
TGAAAGCTACTCTTCATTTAAGACTGCTCTCCCCAACCAAGACACATTTGC
CTGGAAATTCAGTTCTTAGCTTAAAGACTAAAATGCAAGCAAACCCTGCA
ATTCCTGGACCTGATAGTTATATTCATGAGTGAAATTGTGGGGAGTCCAGC
CATTTGGGAGGCAATGACTTTCTGCTGGCCCATGTTTCAGTTGCCAGTAAG
CTTCTCACATTTAATAAAGTGTACTTTTTAGAACATT SEQ ID NO: 90

Spondin 2, Extracellular Matrix Protein

>gi|6912681|ref|NM_012445.1| Homo sapiens spondin 2, extracellular matrix protein (SPON2), mRNA

GCACGAGGGAAGAGGGTGATCCGACCCGGGGAAGGTCGCTGGGCA
GGGCGAGTTGGGAAAGCGGCAGCCCCCGCCGCCCCCGCAGCCCCTTCTCC
TCCTTTCTCCCACGTCCTATCTGCCTCTCGCTGGAGGCCAGGCCGTGCAGC

ATCGAAGACAGGAGGAACTGGAGCCTCATTGGCCGGCCCGGGGCGCCGG
CCTCGGGCTTAAATAGGAGCTCCGGGCTCTGGCTGGGACCCGACCGCTGC
CGGCCGCGCTCCCGCTGCTCCTGCCGGGTGATGGAAAACCCCAGCCCGGC
CGCCGCCCTGGGCAAGGCCCTCTGCGCTCTCCTCCTGGCCACTCTCGGCGC
CGCCGGCCAGCCTCTTGGGGGAGAGTCCATCTGTTCCGCCAGAGCCCCGG
CCAAATACAGCATCACCTTCACGGGCAAGTGGAGCCAGACGGCCTTCCCC
AAGCAGTACCCCCTGTTCCGCCCCCCTGCGCAGTGGTCTTCGCTGCTGGGG
GCCGCGCATAGCTCCGACTACAGCATGTGGAGGAAGAACCAGTACGTCAG
TAACGGGCTGCGCGACTTTGCGGAGCGCGGCGAGGCCTGGGCGCTGATGA
AGGAGATCGAGGCGGCGGGGGGAGGCGCTGCAGAGCGTGCACGCGGTGTT
TTCGGCGCCCGCCGTCCCCAGCGGCACCGGGCAGACGTCGGCGGAGCTGG
AGGTGCAGCGCAGGCACTCGCTGGTCTCGTTTGTGGTGCGCATCGTGCCC
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CCGTTGGCGGGAACAGGCGGCGCTGGACCTGTACCCCTACGACGCCGGGA
CGGACAGCGGCTTCACCTTCTCCTCCCCCAACTTCGCCACCATCCCGCAGG
ACACGGTGACCGAGATAACGTCCTCCTCTCCCAGCCACCCGGCCAACTCC
TTCTACTACCCGCGGCTGAAGGCCCTGCCTCCCATCGCCAGGGTGACACTG
GTGCGGCTGCGACAGAGCCCCAGGGCCTTCATCCCTCCCGCCCCAGTCCT
GCCCAGCAGGGACAATGAGATTGTAGACAGCGCCTCAGTTCCAGAAACGC
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CTCAC SEQ ID NO: 91

Olfactomedin 1, Transcript Variant 3

>gi|34335282|ref|NM_058199.2| Homo sapiens olfactomedin 1 (OLFM1), transcript variant 3, mRNA

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Thrombospondin Repeat Containing 1

>gi|38016903|ref|NM_019032.2| Homo sapiens thrombospondin repeat containing 1 (TSRC1), mRNA

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Thrombospondin 2

>gi|40317627|ref|NM_003247.2| Homo sapiens thrombospondin 2 (THBS2), mRNA | qPCR forward_primer match [3558..3580] | qPCR reverse_primer match [3682..3655] | qPCR probe match [3597..3623]

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Adlican

>gi|18390318|ref|NM_015419.1| Homo sapiens adlican (DKFZp564I1922), mRNA | qPCR assay_on_demand_context match [694..718]

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GTTCTTCCACAACTCTCTCAAGCATAAAAGTGGAGGTGGCTTCAAGTCAG
GCAGAAACCACCACCCTTGATCAAGATCATCTTGAAACCACTGTGGCTAT
TCTCCTTTCTGAAACTAGACCACAGAATCACACCCCTACTGCTGCCCGGAT
GAAGGAGCCAGCATCCTCGTCCCCATCCACAATTCTCATGTCTTTGGGACA
AACCACCACCACTAAGCCAGCACTTCCCAGTCCAAGAATATCTCAAGCAT
CTAGAGATTCCAAGGAAAATGTTTTCTTGAATTATGTGGGGAATCCAGAA
ACAGAAGCAACCCCAGTCAACAATGAAGGAACACAGCATATGTCAGGGC
CAAATGAATTATCAACACCCTCTTCCGACCGGGATGCATTTAACTTGTCTA
CAAAGCTGGAATTGGAAAAGCAAGTATTTGGTAGTAGGAGTCTACCACGT
GGCCCAGATAGCCAACGCCAGGATGGAAGAGTTCATGCTTCTCATCAACT
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CTCGTCACTGGACCAACAAACCGGAAATAACTACATATCCTTCTGGGGCT
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TGAGGCAACAGGAAAACCAAAGCCTTTCGTTACTTGGACAAAGGTTTCCA
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GAGAGGAGAGGAAGGAGAAAACTGCATCATAACTTTACAGAATTGAATC
TAGAGTCTTCCCCGAAAAGCCCAGAAACTTCTCTGCAGTATCTGGCTTGTC
CATCTGGTCTAAGGTGGCTGCTTCTTCCCCAGCCATGAGTCAGTTTGTGCC
CATGAATAATACACGACCTGTTATTTCCATGACTGCTTTACTGTATTTTTA
AGGTCAATATACTGTACATTTGATAATAAAATAATATTCTCCCAAAAAAA
AAA

SEQ ID NO: 95

Cystatin SA

>gi|19882252|ref|NM_001322.2| Homo sapiens cystatin SA (CST2), mRNA | qPCR forward_primer match [302..320] | qPCR reverse_primer match [393..370] | qPCR probe match [341..369]

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TGCTGGCCACCCAGGCTGTGGCCCTGGCCTGGAGCCCCCAGGAGGAGGAC
AGGATAATCGAGGGTGGCATCTATGATGCAGACCTCAATGATGAGCGGGT
ACAGCGTGCCCTTCACTTTGTCATCAGCGAGTATAACAAGGCCACTGAAG
ATGAGTACTACAGACGCCTGCTGCGGGTGCTACGAGCCAGGGAGCAGATC
GTGGGCGGGGTGAATTACTTCTTCGACATAGAGGTGGGCCGAACCATATG
TACCAAGTCCCAGCCCAACTTGGACACCTGTGCCTTCCATGAACAGCCAG
AACTGCAGAAGAAACAGTTGTGCTCTTTCCAGATCTACGAAGTTCCCTGG
GAGGACAGAATGTCCCTGGTGAATTCCAGGTGTCAAGAAGCCTAGGGATC
TGTGCCAGGGAGTCACACTGACCACCTCCTACTCCCACCCCTTGTAGTGCT
CCCACCCCTGGACTGGTGGCCCCCACCCTGTGGGAGGTCTCCCCATGCACC
TGCAGCAGGAGAAGACAGAGAAGGCTGCAGGAGGCCTTTGTTGCTCAGC
AGGGGACTCTGCCCTCCCTCCTTCCTTTTGCTTCTCATAGCCCTGGTACATG
GTACACACACCCCCACCTCCTGCAATTAAACAGTAGCATCACCTC

SEQ ID NO: 96

Cystatin SN

>gi|19882250|ref|NM_001898.2| Homo sapiens cystatin SN (CST1), mRNA | qPCR forward_primer match [358..376] | qPCR reverse_primer match [449..426] | qPCR probe match [397..425]

GGGCTCCCTGCCTCGGGCTCTCACCCTCCTCTCCTGCAGCTCCAGCT
TTGTGCTCTGCCTCTGAGGAGACCATGGCCCAGTATCTGAGTACCCTGCTG
CTCCTGCTGGCCACCCTAGCTGTGGCCCTGGCCTGGAGCCCCAAGGAGGA
GGATAGGATAATCCCGGGTGGCATCTATAACGCAGACCTCAATGATGAGT
GGGTACAGCGTGCCCTTCACTTCGCCATCAGCGAGTATAACAAGGCCACC
AAAGATGACTACTACAGACGTCCGCTGCGGGTACTAAGAGCCAGGCAACA
GACCGTTGGGGGGGTGAATTACTTCTTCGACGTAGAGGTGGGCCGCACCA
TATGTACCAAGTCCCAGCCCAACTTGGACACCTGTGCCTTCCATGAACAGC
CAGAACTGCAGAAGAAACAGTTGTGCTCTTTCGAGATCTACGAAGTTCCC
TGGGAGAACAGAAGGTCCCTGGTGAAATCCAGGTGTCAAGAATCCTAGGG
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TGCTCCCACCCCTGGACTGGTGGCCCCCACCCTGCGGGAGGCCTCCCCATG
TGCCTGCGCCAAGAGACAGACAGAGAAGGCTGCAGGAGTCCTTTGTTGCT
CAGCAGGGCGCTCTGCCCTCCCTCCTTCCTTCTTGCTTCTAATAGCCCTGGT
ACATGGTACACACCCCCCCACCTCCTGCAATTAAACAGTAGCATCGCCTCC
CTCTGAAAAAAAAAAAAAAAAAAAAA SEQ ID NO: 97

Lysyl Oxidase-Like Enzyme 2

>gi|4505010|ref|NM_002318.1| Homo sapiens lysyl oxidase-like 2 (LOXL2), mRNA | qPCR forward_primer match [2205..2223] | qPCR reverse_primer match [2286..2269] | qPCR probe match [2261..2229]

ACTCCAGCGCGCGGCTACCTACGCTTGGTGCTTGCTTTCTCCAGCCA
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GGAAGCGGAGGGGGACCACCGTGGAGAGCGCGGTCCCAGCCCGGCCACT
GCGGATCCCTGAAACCAAAAAGCTCCTGCTGCTTCTGTACCCCGCCTGTCC
CTCCCAGCTGCGCAGGGCCCCTTCGTGGGATCATCAGCCCGAAGACAGGG
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CTCCTGTCCCCCCTGAGCCTGGCACAGTATGACAGCTGGCCCCATTACCCC
GAGTACTTCCAGCAACCGGCTCCTGAGTATCACCAGCCCCAGGCCCCCGC
CAACGTGGCCAAGATTCAGCTGCGCCTGGCTGGGCAGAAGAGGAAGCAC
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GCTATGTGGAGGCCAAGTCCTGGACTGCCAGCTCCTCCTACGGCAAGGGA
GAAGGGCCCATCTGGTTAGACAATCTCCACTGTACTGGCAACGAGGCGAC
CCTTGCAGCATGCACCTCCAATGGCTGGGGCGTCACTGACTGCAAGCACA
CGGAGGATGTCGGTGTGGTGTGCAGCGACAAAAGGATTCCTGGGTTCAAA
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TGATGGAGGGCTACGTGGAGGTGAAGGAGGGCAAGACCTGGAAGCAGAT
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ACCCCATGAAGAATGTCACCTGCGAGAATGGGCTGCCGGCCGTGGTGAGT
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AACTTGGAATAAAAGAAAGATTTATG SEQ ID NO: 98

Thyroglobulin

>gi|33589851|ref|NM_003235.3| Homo sapiens thyroglobulin (TG), mRNA | qPCR forward_primer match [886..905] | qPCR reverse_primer match [962..941] | qPCR probe match [915..939]

GCAGTGGTTTCTCCTCCTTCCTCCCAGGAAGGGCCAGGAAAATGGC
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TGCTGGTGTGTGGGTGCCAACGGCAGTGAAGTGCTGGGCAGCAGGCAGCC
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CAGTGCTGGTGTGTGGACGCAGAGGGGATGGAGGTGTATGGGACCCGCCA
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GGGAGAGTTTATGCCTGTCCAGTGCAAATTTGTCAACACCACAGACATGA
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CTATGGGATGTAGAAAAAACACAGTGCCAAGGCCAGCATCTCCAACAGA
AGCAGGTTTGACAACAGAACTTTTCTCCCCTGTGGACCTCAACCAGGTCAT
TGTCAATGGAAATCAATCACTATCCAGCCAGAAGCACTGGCTTTTCAAGC
ACCTGTTTTCAGCCCAGCAGGCAAACCTATGGTGCCTTTCTCGTTGTGTGC
AGGAGCACTCTTTCTGTCAGCTCGCAGAGATAACAGAGAGTGCATCCTTG
TACTTCACCTGCACCCTCTACCCAGAGGCACAGGTGTGTGATGACATCATG
GAGTCCAATACCCAGGGCTGCAGACTGATCCTGCCTCAGATGCCAAAGGC
CCTGTTCCGGAAGAAAGTTATACTGGAAGATAAAGTGAAGAACTTTTACA
CTCGCCTGCCGTTCCAAAAACTGATGGGGATATCCATTAGAAATAAAGTG
CCCATGTCTGAAAAATCTATTTCTAATGGGTTCTTTGAATGTGAACGACGG
TGCGATGCGGACCCATGCTGCACTGGCTTTGGATTTCTAAATGTTTCCCAG
TTAAAAGGAGGAGAGGTGACATGTCTCACTCTGAACAGCTTGGGAATTCA
GATGTGCAGTGAGGAGAATGGAGGAGCCTGGCGCATTTTGGACTGTGGCT
CTCCTGACATTGAAGTCCACACCTATCCCTTCGGATGGTACCAGAAGCCCA
TTGCTCAAAATAATGCTCCCAGTTTTTGCCCTTTGGTTGTTCTGCCTTCCCT
CACAGAGAAAGTGTCTCTGGAATCGTGGCAGTCCCTGGCCCTCTCTTCAGT
GGTTGTTGATCCATCCATTAGGCACTTTGATGTTGCCCATGTCAGCACTGC
TGCCACCAGCAATTTCTCTGCTGTCCGAGACCTCTGTTTGTCGGAATGTTC
CCAACATGAGGCCTGTCTCATCACCACTCTGCAAACCCAACTCGGGGCTG
TGAGATGTATGTTCTATGCTGATACTCAAAGCTGCACACATAGTCTGCAGG
GTCGGAACTGCCGACTTCTGCTTCGTGAAGAGGCCACCCACATCTACCGG
AAGCCAGGAATCTCTCTGCTCAGCTATGAGGCATCTGTACCTTCTGTGCCC
ATTTCCACCCATGGCCGGCTGCTGGGCAGGTCCCAGGCCATCCAGGTGGG
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GCCCCTGGCAGAGAGGCACTTCCAGGCACCAGAGCCCTTGAACTGGACAG
GCTCCTGGGATGCCAGCAAGCCAAGGGCCAGCTGCTGGCAGCCAGGCACC
AGAACATCCACGTCTCCTGGAGTCAGTGAAGATTGTTTGTATCTCAATGTG
TTCATCCCTCAGAATGTGGCCCCTAACGCGTCTGTGCTGGTGTTCTTCCAC
AACACCATGGACAGGGAGGAGAGTGAAGGATGGCCGGCTATCGACGGCT
CCTTCTTGGCTGCTGTTGGCAACCTCATCGTGGTCACTGCCAGCTACCGAG
TGGGTGTCTTCGGCTTCCTGAGTTCTGGATCCGGAGAGGTGAGTGGCAACT
GGGGGCTGCTGGACCAGGTGGCGGCTCTGACCTGGGTGCAGACCCACATC
CGAGGATTTGGCGGGGACCCTCGGCGCGTGTCCCTGGCAGCAGACCGTGG
CGGGGCTGATGTGGCCAGCATCCACCTTCTCACGGCCAGGGCCACCAACT

CCCAACTTTTCCGGAGAGCTGTGCTGATGGGAGGCTCCGCACTCTCCCCGG
CCGCCGTCATCAGCCATGAGAGGGCTCAGCAGCAGGCAATTGCTTTGGCA
AAGGAGGTCAGTTGCCCCATGTCATCCAGCCAAGAAGTGGTGTCCTGCCT
CCGCCAGAAGCCTGCCAATGTCCTCAATGATGCCCAGACCAAGCTCCTGG
CCGTGAGTGGCCCTTTCCACTACTGGGGTCCTGTGATCGATGGCCACTTCC
TCCGTGAGCCTCCAGCCAGAGCACTGAAGAGGTCTTTATGGGTAGAGGTC
GATCTGCTCATTGGGAGTTCTCAGGACGACGGGCTCATCAACAGAGCAAA
GGCTGTGAAGCAATTTGAGGAAAGTCGAGGCCGGACCAGTAGCAAAACA
GCCTTTTACCAGGCACTGCAGAATTCTCTGGGTGGCGAGGACTCAGATGC
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TATCATCTGCCCTATAATCGACATGGCCAGTGCCTGGGCAAAGAGGGCCC
GAGGAAACGTCTTCATGTACCATGCTCCTGAAAACTACGGCCATGGCAGC
CTGGAGCTGCTGGCGGATGTTCAGTTTGCCTTGGGGCTTCCCTTCTACCCA
GCCTACGAGGGGCAGTTTTCTCTGGAGGAGAAGAGCCTGTCGCTGAAAAT
CATGCAGTACTTTTCCCACTTCATCAGATCAGGAAATCCCAACTACCCTTA
TGAGTTCTCACGGAAAGTACCCACATTTGCAACCCCCTGGCCTGACTTTGT
ACCCCGTGCTGGTGGAGAGAACTACAAGGAGTTCAGTGAGCTGCTCCCCA
ATCGACAGGGCCTGAAGAAAGCCGACTGCTCCTTCTGGTCCAAGTACATC
TCGTCTCTGAAGACATCTGCAGATGGAGCCAAGGGCGGGCAGTCAGCAGA
GAGTGAAGAGGAGGAGTTGACGGCTGGATCTGGGCTAAGAGAAGATCTC
CTAAGCCTCCAGGAACCAGGCTCTAAGACCTACAGCAAGTGACCAGCCCT
TGAGCTCCCCAAAAACCTCACCCGAGGCTGCCCACTATGGTCATCTTTTTC
TCTAAAATAGTTACTTACCTTCAATAAAGTATCTACATGCGGTG

SEQ ID NO: 99

Transforming Growth Factor, Beta 1

>gi|10863872|ref|NM_000660.1| Homo sapiens transforming growth factor, beta 1 (Camurati-Engelmann disease) (TGFB1), mRNA | qPCR forward_primer match [1651..1668] | qPCR reverse_primer match [1539..1557] | qPCR probe match [1687..1713]

ACCTCCCTCCGCGGAGCAGCCAGACAGCGAGGGCCCCGGCCGGGG
GCAGGGGGGACGCCCCGTCCGGGGCACCCCCCCCGGCTCTGAGCCGCCCG
CGGGGCCGGCCTCGGCCCGGAGCGGAGGAAGGAGTCGCCGAGGAGCAGC
CTGAGGCCCCAGAGTCTGAGACGAGCCGCCGCCGCCCCCGCCACTGCGGG
GAGGAGGGGGAGGAGGAGCGGGAGGAGGGACGAGCTGGTCGGGAGAAG
AGGAAAAAAAACTTTTGAGACTTTTCCGTTGCCGCTGGGAGCCGGAGGCGC
GGGGACCTCTTGGCGCGACGCTGCCCCGCGAGGAGGCAGGACTTGGGGAC
CCCAGACCGCCTCCCTTTGCCGCCGGGGACGCTTGCTCCCTCCCTGCCCCC
TACACGGCGTCCCTCAGGCGCCCCCATTCCGGACCAGCCCTCGGGAGTCG
CCGACCCGGCCTCCCGCAAAGACTTTTCCCCAGACCTCGGGCGCACCCCCT
GCACGCCGCCTTCATCCCCGGCCTGTCTCCTGAGCCCCCGCGCATCCTAGA
CCCTTTCTCCTCCAGGAGACGGATCTCTCTCCGACCTGCCACAGATCCCCT
ATTCAAGACCACCCACCTTCTGGTACCAGATCGCGCCCATCTAGGTTATTT
CCGTGGGATACTGAGACACCCCCGGTCCAAGCCTCCCCTCCACCACTGCG
CCCTTCTCCCTGAGGAGCCTCAGCTTTCCCTCGAGGCCCTCCTACCTTTTGC
CGGGAGACCCCCAGCCCCTGCAGGGGCGGGGCCTCCCCACCACACCAGCC

CTGTTCGCGCTCTCGGCAGTGCCGGGGGGCGCCGCCTCCCCCATGCCGCCC
TCCGGGCTGCGGCTGCTGCCGCTGCTGCTACCGCTGCTGTGGCTACTGGTG
CTGACGCCTGGCCCGCCGGCCGCGGGACTATCCACCTGCAAGACTATCGA
CATGGAGCTGGTGAAGCGGAAGCGCATCGAGGCCATCCGCGGCCAGATCC
TGTCCAAGCTGCGGCTCGCCAGCCCCCCGAGCCAGGGGGAGGTGCCGCCC
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GGTGGCCGGGGAGAGTGCAGAACCGGAGCCCGAGCCTGAGGCCGACTAC
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CTATGACAAGTTCAAGCAGAGTACACACAGCATATATATGTTCTTCAACA
CATCAGAGCTCCGAGAAGCGGTACCTGAACCCGTGTTGCTCTCCCGGGCA
GAGCTGCGTCTGCTGAGGAGGCTCAAGTTAAAAGTGGAGCAGCACGTGGA
GCTGTACCAGAAATACAGCAACAATTCCTGGCGATACCTCAGCAACCGGC
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TTGTGCGGCAGTGGTTGAGCCGTGGAGGGGAAATTGAGGGCTTTCGCCTT
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CAACGGGTTCACTACCGGCCGCCGAGGTGACCTGGCCACCATTCATGGCA
TGAACCGGCCTTTCCTGCTTCTCATGGCCACCCCGCTGGAGAGGGCCCAGC
ATCTGCAAAGCTCCCGGCACCGCCGAGCCCTGGACACCAACTATTGCTTC
AGCTCCACGGAGAAGAACTGCTGCGTGCGGCAGCTGTACATTGACTTCCG
CAAGGACCTCGGCTGGAAGTGGATCCACGAGCCCAAGGGCTACCATGCCA
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GCAAGGTCCTGGCCCTGTACAACCAGCATAACCCGGGCGCCTCGGCGGCG
CCGTGCTGCGTGCCGCAGGCGCTGGAGCCGCTGCCCATCGTGTACTACGT
GGGCCGCAAGCCCAAGGTGGAGCAGCTGTCCAACATGATCGTGCGCTCCT
GCAAGTGCAGCTGAGGTCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG
GCCCCACCCCGCCCCGCCCCCGCTGCCTTGCCCATGGGGGCTGTATTTAAG
GACACCGTGCCCCAAGCCCACCTGGGGCCCCATTAAAGATGGAGAGAGG
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GTTCCCCCACTCCCACTCCCTCTCTCTCCCTCTCTGCCTCCTCCTGCCTGTC
TGCACTATTCCTTTGCCCGGCATCAAGGCACAGGGGACCAGTGGGGAACA
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TTACATTAATGAACTCATTCAGTCACCATAGCAACACTCTGAGATGGCAG
GGACTCTGATAACACCCATTTTAAAGGTTGAGGAAACAAGCCCAGAGAGG
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GAAGAAGACAATAAAAGATAGTAGTTCAGGCCAGGCGGGGTGCTCACGC
CTGTAATCCTAGCACTTTTGGGAGGCAGAGATGGGAGGATACTTGAATCC
AGGCATTTGAGACCAGCCTGGGTAACATAGTGAGACCCTATCTCTACAAA
ACACTTTTAAAAAATGTACACCTGTGGTCCCAGCTACTCTGGAGGCTAAG
GTGGGAGGATCACTTGATCCTGGGAGGTCAAGGCTGCAG

SEQ ID NO: 100

Serine Proteinase Inhibitor, Clade H, Member 1

>gi|32454740|ref|NM_001235.2| Homo sapiens serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1) (SERPINH1), mRNA | qPCR assay_on_demand_context match [184..208]

TCTTTGGCTTTTTTTGGCGGAGCTGGGGCGCCCTCCGGAAGCGTTTC
CAACTTTCCAGAAGTTTCTCGGGACGGGCAGGAGGGGGTGGGGACTGCCA

TATATAGATCCCGGGAGCAGGGGAGCGGGCTAAGAGTAGAATCGTGTCGC
GGCTCGAGAGCGAGAGTCACGTCCCGGCGCTAGCCCAGCCCGACCCAGGC
CCACCGTGGTGCACGCAAACCACTTCCTGGCCATGCGCTCCCTCCTGCTTC
TCAGCGCCTTCTGCCTCCTGGAGGCGGCCCTGGCCGCCGAGGTGAAGAAA
CCTGCAGCCGCAGCAGCTCCTGGCACTGCGGAGAAGTTGAGCCCCAAGGC
GGCCACGCTTGCCGAGCGCAGCGCCGGCCTGGCCTTCAGCTTGTACCAGG
CCATGGCCAAGGACCAGGCAGTGGAGAACATCCTGGTGTCACCCGTGGTG
GTGGCCTCGTCGCTAGGGCTCGTGTCGCTGGGCGGCAAGGCGACCACGGC
GTCGCAGGCCAAGGCAGTGCTGAGCGCCGAGCAGCTGCGCGACGAGGAG
GTGCACGCCGGCCTGGGCGAGCTGCTGCGCTCACTCAGCAACTCCACGGC
GCGCAACGTGACCTGGAAGCTGGGCAGCCGACTGTACGGACCCAGCTCAG
TGAGCTTCGCTGATGACTTCGTGCGCAGCAGCAAGCAGCACTACAACTGC
GAGCACTCCAAGATCAACTTCCGCGACAAGCGCAGCGCGCTGCAGTCCAT
CAACGAGTGGGCCGCGCAGACCACCGACGGCAAGCTGCCCGAGGTCACC
AAGGACGTGGAGCGCACGGACGGCGCCCTGCTAGTCAACGCCATGTTCTT
CAAGCCACACTGGGATGAGAAATTCCACCACAAGATGGTGGACAACCGTG
GCTTCATGGTGACTCGGTCCTATACCGTGGGTGTCATGATGATGCACCGGA
CAGGCCTCTACAACTACTACGACGACGAGAAGGAAAAGCTGCAAATCGTG
GAGATGCCCCTGGCCCACAAGCTCTCCAGCCTCATCATCCTCATGCCCCAT
CACGTGGAGCCTCTCGAGCGCCTTGAAAAGCTGCTAACCAAAGAGCAGCT
GAAGATCTGGATGGGGAAGATGCAGAAGAAGGCTGTTGCCATCTCCTTGC
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GTCAGGCAAGAAGGACCTGTACCTGGCCAGCGTGTTCCACGCCACCGCCT
TTGAGTTGGACACAGATGGCAACCCCTTTGACCAGGACATCTACGGGCGC
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AGCTAGAATTCACTCCACTTGGACATGGGCCCCAGATACCATGATGCTGA
GCCCGGAAACTCCACATCCTGTGGGACCTGGGCCATAGTCATTCTGCCTGC
CCTGAAAGTCCCAGATCAAGCCTGCCTCAATCAGTATTCATATTTATAGCC
AGGTACCTTCTCACCTGTGAGACCAAATTGAGCTAGGGGGGTCAGCCAGC
CCTCTTCTGACACTAAAACACCTCAGCTGCCTCCCCAGCTCTATCCCAACC
TCTCCCAACTATAAAACTAGGTGCTGCAGCCCCTGGGACCAGGCACCCCC
AGAATGACCTGGCCGCAGTGAGGCGGATTGAGAAGGAGCTCCCAGGAGG
GGCTTCTGGGCAGACTCTGGTCAAGAAGCATCGTGTCTGGCGTTGTGGGG
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GAAGGGGGAACATGAGCCTTTGTTGCTATCAATCCAAGAACTTATTTGTA
CATTTTTTTTTTCAATAAAACTTTTCCAATGACATTTTGTTGGAGCGTGGAA
AAAA SEQ ID NO: 101

Serine Proteinase Inhibitor, Clade B, Member 5

>gi|4505788|ref|NM_002639.1| Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA | qPCR

forward_primer match [36..56] | qPCR reverse_primer match [106..86] | qPCR probe match [60..80]

GGCACGAGTTGTGCTCCTCGCTTGCCTGTTCCTTTTCCACGCATTTT
CCAGGATAACTGTGACTCCAGGCCCGCAATGGATGCCCTGCAACTAGCAA
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TGGGCAATGTCCTCTTCTCTCCAATCTGTCTCTCCACCTCTCTGTCACTTGC
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ATTTTGAAAATGTCAAAGATATACCCTTTGGATTTCAAACAGTAACATCGG
ATGTAAACAAACTTAGTTCCTTTTACTCACTGAAACTAATCAAGCGGCTCT
ACGTAGACAAATCTCTGAATCTTTCTACAGAGTTCATCAGCTCTACGAAGA
GACCCTATGCAAAGGAATTGGAAACTGTTGACTTCAAAGATAAATTGGAA
GAAACGAAAGGTCAGATCAACAACTCAATTAAGGATCTCACAGATGGCCA
CTTTGAGAACATTTTAGCTGACAACAGTGTGAACGACCAGACCAAAATCC
TTGTGGTTAATGCTGCCTACTTTGTTGGCAAGTGGATGAAGAAATTTCCTG
AATCAGAAACAAAAGAATGTCCTTTCAGACTCAACAAGACAGACACCAA
ACCAGTGCAGATGATGAACATGGAGGCCACGTTCTGTATGGGAAACATTG
ACAGTATCAATTGTAAGATCATAGAGCTTCCTTTTCAAAATAAGCATCTCA
GCATGTTCATCCTACTACCCAAGGATGTGGAGGATGAGTCCACAGGCTTG
GAGAAGATTGAAAAACAACTCAACTCAGAGTCACTGTCACAGTGGACTAA
TCCCAGCACCATGGCCAATGCCAAGGTCAAACTCTCCATTCCAAAATTTA
AGGTGGAAAAGATGATTGATCCCAAGGCTTGTCTGGAAAATCTAGGGCTG
AAACATATCTTCAGTGAAGACACATCTGATTTCTCTGGAATGTCAGAGAC
CAAGGGAGTGGCCCTATCAAATGTTATCCACAAAGTGTGCTTAGAAATAA
CTGAAGATGGTGGGGATTCCATAGAGGTGCCAGGAGCACGGATCCTGCAG
CACAAGGATGAATTGAATGCTGACCATCCCTTTATTTACATCATCAGGCAC
AACAAAACTCGAAACATCATTTTCTTTGGCAAATTCTGTTCTCCTTAAGTG
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TAGACCTTTTTTTTTTTCCAATTCTATCTTTTGTTTCCTTTTTTCCCATAAGA
CAATGACATACGCTTTTAATGAAAAGGAATCACGTTAGAGGAAAAATATT
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CACAAAGAAAATTCCTATAAGGAAGATTTGGAAGCTCTTCTTCCCAGCAC
TATGCTTTCCTTCTTTGGGATAGAGAATGTTCCAGACATTCTCGCTTCCCTG
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CAGTGAAACTTGGGCACATGCTCAGGCTACTATAGGTCCAGAAGTCCTTA
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GGGGTCAAAATTTGCTGCCAAATGCGTATGCCACCAACTTACAAAAACAC
TTCGTTCGCAGAGCTTTTCAGATTGTGGAATGTTGGATAAGGAATTATAGA
CCTCTAGTAGCTGAAATGCAAGACCCCAAGAGGAAGTTCAGATCTTAATA
TAAATTCACTTTCATTTTTGATAGCTGTCCCATCTGGTCATGTGGTTGGCAC
TAGACTGGTGGCAGGGGCTTCTAGCTGACTCGCACAGGGATTCTCACAAT
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TGATTAAAGTGCTCACGTTACCTTGACACATAGTTTTTCAGTCTATGGGTT
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GTTGGGTGGATAAGCTATCCCTGTTGCCGGTTCATGGATTACTTCTCTATA
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CCTTGACATGCATTGTAAATAGGTTCTTCTTGTTCTGAGATTCAATATTGA
ATTTCTCCTATGCTATTGACAATAAAATATTATTGAACTACC

SEQ ID NO: 102

Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5

>gi|11386170|ref|NM_004363.1| Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), mRNA | qPCR assay_on_demand_context match [2128..2152]

CTCAGGGCAGAGGGAGGAAGGACAGCAGACCAGACAGTCACAGC
AGCCTTGACAAAACGTTCCTGGAACTCAAGCTCTTCTCCACAGAGGAGGA
CAGAGCAGACAGCAGAGACCATGGAGTCTCCCTCGGCCCCTCCCCACAGA
TGGTGCATCCCCTGGCAGAGGCTCCTGCTCACAGCCTCACTTCTAACCTTC
TGGAACCCGCCCACCACTGCCAAGCTCACTATTGAATCCACGCCGTTCAAT
GTCGCAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAATCTGCCCCAGCA
TCTTTTTGGCTACAGCTGGTACAAAGGTGAAAGAGTGGATGGCAACCGTC
AAATTATAGGATATGTAATAGGAACTCAACAAGCTACCCCAGGGCCCGCA
TACAGTGGTCGAGAGATAATATACCCCAATGCATCCCTGCTGATCCAGAA
CATCATCCAGAATGACACAGGATTCTACACCCTACACGTCATAAAGTCAG
ATCTTGTGAATGAAGAAGCAACTGGCCAGTTCCGGGTATACCCGGAGCTG
CCCAAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAGGA
TGCTGTGGCCTTCACCTGTGAACCTGAGACTCAGGACGCAACCTACCTGTG
GTGGGTAAACAATCAGAGCCTCCCGGTCAGTCCCAGGCTGCAGCTGTCCA
ATGGCAACAGGACCCTCACTCTATTCAATGTCACAAGAAATGACACAGCA
AGCTACAAATGTGAAACCCAGAACCCAGTGAGTGCCAGGCGCAGTGATTC
AGTCATCCTGAATGTCCTCTATGGCCCGGATGCCCCCACCATTTCCCCTCT
AAACACATCTTACAGATCAGGGGAAAATCTGAACCTCTCCTGCCACGCAG
CCTCTAACCCACCTGCACAGTACTCTTGGTTTGTCAATGGGACTTTCCAGC
AATCCACCCAAGAGCTCTTTATCCCCAACATCACTGTGAATAATAGTGGAT
CCTATACGTGCCAAGCCCATAACTCAGACACTGGCCTCAATAGGACCACA
GTCACGACGATCACAGTCTATGCAGAGCCACCCAAACCCTTCATCACCAG
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CTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGACAACAGGACCCTCAC
TCTACTCAGTGTCACAAGGAATGATGTAGGACCCTATGAGTGTGGAATCC
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GGGGTGAACCTCAGCCTCTCCTGCCATGCAGCCTCTAACCCACCTGCACA
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TGGAGGACAAGGATGCTGTGGCCTTCACCTGTGAACCTGAGGCTCAGAAC
ACAACCTACCTGTGGTGGGTAAATGGTCAGAGCCTCCCAGTCAGTCCCAG
GCTGCAGCTGTCCAATGGCAACAGGACCCTCACTCTATTCAATGTCACAA
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AACCGCAGTGACCCAGTCACCCTGGATGTCCTCTATGGGCCGGACACCCC
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TCTGATACCACTGCACTGTCTGAGAATTTCCAAAACTTTAATGAACTAACT
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AACAGAAAAA SEQ ID NO: 103

Matrix Metalloproteinase 2

>gi|11342665|ref|NM_004530.1| Homo sapiens matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) (MMP2), mRNA | qPCR forward_primer match [1713..1732] | qPCR reverse_primer match [1793..1775] | qPCR probe match [1751..1773]

TGTTTCCGCTGCATCCAGACTTCCTCAGGCGGTGGCTGGAGGCTGC
GCATCTGGGGCTTTAAACATACAAAGGGATTGCCAGGACCTGCGGCGGCG
GCGGCGGCGGCGGGGGCTGGGGCGCGGGGGCCGGACCATGAGCCGCTGA
GCCGGGCAAACCCCAGGCCACCGAGCCAGCGGACCCTCGGAGCGCAGCC
CTGCGCCGCGGACCAGGCTCCAACCAGGCGGCGAGGCGGCCACACGCAC
CGAGCCAGCGACCCCCGGGCGACGCGCGGGGCCAGGGAGCGCTACGATG
GAGGCGCTAATGGCCCGGGGCGCGCTCACGGGTCCCCTGAGGGCGCTCTG
TCTCCTGGGCTGCCTGCTGAGCCACGCCGCCGCCGCGCCGTCGCCCATCAT
CAAGTTCCCCGGCGATGTCGCCCCCAAAACGGACAAAGAGTTGGCAGTGC
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GTGCTGAAGGACACACTAAAGAAGATGCAGAAGTTCTTTGGACTGCCCCA
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TGGGACAAGAACCAGATCACATACAGGATCATTGGCTACACACCTGATCT
GGACCCAGAGACAGTGGATGATGCCTTTGCTCGTGCCTTCCAAGTCTGGA
GCGATGTGACCCCACTGCGGTTTTCTCGAATCCATGATGGAGAGGCAGAC
ATCATGATCAACTTTGGCCGCTGGGAGCATGGCGATGGATACCCCTTTGA
CGGTAAGGACGGACTCCTGGCTCATGCCTTCGCCCCAGGCACTGGTGTTG

GGGGAGACTCCCATTTTGATGACGATGAGCTATGGACCTTGGGAGAAGGC
CAAGTGGTCCGTGTGAAGTATGGCAACGCCGATGGGGAGTACTGCAAGTT
CCCCTTCTTGTTCAATGGCAAGGAGTACAACAGCTGCACTGATACTGGCC
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CGCTGAAGGACAGCCCTGCAAGTTTCCATTCCGCTTCCAGGGCACATCCTA
TGACAGCTGCACCACTGAGGGCCGCACGGATGGCTACCGCTGGTGCGGCA
CCACTGAGGACTACGACCGCGACAAGAAGTATGGCTTCTGCCCTGAGACC
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TTCACTTTCCTGGGCAACAAATATGAGAGCTGCACCAGCGCCGGCCGCAG
TGACGGAAAGATGTGGTGTGCGACCACAGCCAACTACGATGACGACCGCA
AGTGGGGCTTCTGCCCTGACCAAGGGTACAGCCTGTTCCTCGTGGCAGCC
CACGAGTTTGGCCACGCCATGGGGCTGGAGCACTCCCAAGACCCTGGGGC
CCTGATGGCACCCATTTACACCTACACCAAGAACTTCCGTCTGTCCCAGGA
TGACATCAAGGGCATTCAGGAGCTCTATGGGGCCTCTCCTGACATTGACCT
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TCTTCAAGGACCGGTTCATTTGGCGGACTGTGACGCCACGTGACAAGCCC
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AAGCCACTGACCAGCCTGGGACTGCCCCCTGATGTCCAGCGAGTGGATGC
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TGAGTGGC SEQ ID NO: 104

Proprotein Convertase Subtilisin/Kexin Type 5

>gi|20336245|ref|NM_006200.2| Homo sapiens proprotein convertase subtilisin/kexin type 5 (PCSK5), mRNA | qPCR forward_primer match [2677..2697] | qPCR reverse_primer match [2821..2801] | qPCR probe match [2737..2765]

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SEQ ID NO: 105

Carboxypeptidase N, polypeptide 2, 83kD

>gi|18554966|ref|XM_087358.1| Homo sapiens carboxypeptidase N, polypeptide 2, 83kD (CPN2), mRNA

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Hyaluronan and proteoglycan link protein 4

>gi|30794471|ref|NM_023002.1| Homo sapiens hyaluronan and proteoglycan link protein 4 (HAPLN4), mRNA

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TCAATAAATCCTTGT SEQ ID NO: 107

Immunohistochemistry

8uM frozen sections were cut from tissue blocks and mounted onto APES slides. The tissue was then fixed in acetone for 10 minutes before being air-dried. The slides were then soaked in 0.3% hydrogen peroxide in methanol for 10 minutes and washed in phosphate-buffered saline (PBS). Non-specific binding sites were blocked by incubating the slides in 20% serum from the appropriate animal and washing again in PBS. Primary antibody diluted in PBS containing 1% serum was then added to the slides. After incubation for 1 hour, the slides were again washed in PBS before incubating with the secondary antibody for a further 1 hour. After final washing in

PBS, the secondary antibody was detected with diaminobenzidine tetrahydrochloride dissolved in Tris buffered saline (TBS), before being washed in TBS and water. The slides were then counter stained in haemotoxylin and viewed under a light microscope.

In certain embodiments, gastric tumors can be localized *in situ* using stains based on cancer markers of this invention. At least one marker may be forming amyloid structures that can be visualized using Congo red or equivalent, non-specific amyloid stains.

Tests for Gastric Cancer Markers in Body Fluids

In several embodiments, assays for GTM can be desirably carried out on samples obtained from blood, plasma, serum, peritoneal fluid obtained for example using peritoneal washes, or other body fluids, such as urine, lymph, cerebrospinal fluid, gastric fluid or stool samples.

In general, methods for assaying for oligonucleotides, proteins and peptides in these fluids are known in the art. Detection of oligonucleotides can be carried out using hybridization methods such as Northern blots, Southern blots or microarray methods, or qPCR. Methods for detecting proteins include such as enzyme linked immunosorbent assays (ELISA), protein chips having antibodies, suspension beads radioimmunoassay (RIA), Western blotting and lectin binding. However, for purposes of illustration, fluid levels of a GTM can be quantified using a sandwich-type enzyme-linked immunosorbent assay (ELISA). For plasma assays, a 5 uL aliquot of a properly diluted sample or serially diluted standard GTM and 75 uL of peroxidase-conjugated anti-human GTM antibody are added to wells of a microtiter plate. After a 30 minute incubation period at 30°C, the wells are washed with 0.05% Tween 20 in phosphate-buffered saline (PBS) to remove unbound antibody. Bound complexes of GTM and anti-GTM antibody are then incubated with o-phenylendiamine containing H_2O_2 for 15 minutes at 30°C. The reaction is stopped by adding 1 M H_2SO_4, and the absorbance at 492 nm is measured with a microtiter plate reader.

It can be appreciated that anti-GTM antibodies can be monoclonal antibodies or polyclonal antisera. It can also be appreciated that any other body fluid can be suitably studied.

Certain markers are known to be present in plasma or serum. These include osteopontin (Hotte et al., Cancer 95(3): 507-510 (2002)), prostate-specific antigen

(Martin et al., Prostate Cancer Prostatic Dis. (March 9, 2004) (Pub Med No: PMID: 15007379), thyroglobulin (Hall et al., Laryngoscope 113(1):77-81 (2003); Mazzaferri et al., J. Clin. Endocrinol. Metab. 88(4):1433-14421 (2003), matrix metalloproteinase-2 and –9 (Kuo et al., Clin. Chem. Acta. 294(1-2):157-168 (2000), CEA and TIMP1 (Pellegrini et al., Cancer Immunol. Immunother. 49(7):388-394 (2000). Thus, because some of the above markers are also useful markers for GTM, plasma, serum or other fluid assays are already available for their detection and quantification. Because many proteins are either (1) secreted by cells, (2) sloughed from cell membranes, or (3) are lost from cells upon cell death, other GTM are also present in body fluids, such as plasma, serum and the like. Therefore, in embodiments of this invention, detection of GTM in conveniently obtained samples will be useful and desirable and can be a basis for diagnosis of gastric cancer.

Western Analysis

Proteins were extracted from gastric tissue using a TriReagent and guanidine HCl extraction method. The non-aqueous phase from the TriReagent extraction of RNA was mixed with 1.5vols of ethanol and centrifuged to remove DNA and OCT medium. 0.5mls of supernatant was mixed with 0.75ml isopropanol, incubated at room temperature for 10 minutes, and then centrifuged. The pellet was washed three times in 1ml 0.3M guanidine HCl in 95% ethanol and once in ethanol alone, then resuspended in 50ul 1% SDS.

Proteins were quantified and electrophoresed on SDS polyacrylamide gels using standard methods. Briefly, the separated proteins were transferred to PVDF membrane using the BioRad trans-blot electrophoretic transfer cell using standard methodology. The membranes were then blocked with a solution containing non-fat milk powder for 30 minutes before being incubated with primary antibody for 2 hours at room temperature. After washing, the membrane was incubated with secondary antibody for 1 hour at room temperature. After final washes, bound antibody was visualized using the ECL detection system (Amersham Biosciences).

Detection of markers in the serum can be accomplished by providing a sample of serum using known methods and then subjecting the serum sample to analysis, either using oligonucleotide probes or antibodies directed against the protein of interest. Immunoblotting, including Western blotting analysis can be especially useful to determine whether alternatively expressed proteins are present in the serum.

Additionally, other body fluids may contain markers, and include peritoneal fluid, cerebrospinal fluid and the like. It is not necessary for a marker to be secreted, in a physiological sense, to be useful. Rather, any mechanism by which a marker protein or gene enters the serum can be effective in producing a detectable, quantifiable level of the marker. Thus, normal secretion of soluble proteins from cells, sloughing of membrane proteins from plasma membranes, secretion of alternatively spliced forms of mRNA or proteins expressed therefrom, cell death (either apoptotic) can produce sufficient levels of the marker to be useful. There is increasing support for the use of serum markers as tools to diagnose and/or evaluate efficacy of therapy for a variety of cancer types.

Yoshikawa et al., (Cancer Letters, 151: 81-86 (2000) describes tissue inhibitor of matrix metalloproteinase-1 in plasma of patients with gastric cancer.

Rudland et al., (Cancer Research 62: 3417-3427 (2002) describes osteopontin as a metastasis associated protein in human breast cancer.

Buckhaults et al., (Cancer Research 61:6996-7001 (2002) describes certain secreted and cell surface genes expressed in colorectal tumors.

Kim et al., (JAMA 287(13):1671-1679 (2002) describes osteopontin as a potential diagnostic biomarker for ovarian cancer.

Hotte et al., (AJ. American Cancer Society 95(3):507-512 (2002) describes plasma osteopontin as a protein detectable in human body fluids and is associated with certain malignancies.

Martin et al., (Prostate Cancer Prostatic Dis. March 9, 2004 (PMID: 15007379) (Abstract) described use of human kallikrein 2, prostate-specific antigen (PSA) and free PSA as markers for detection of prostate cancer.

Hall et al (Laryngoscope 113(1):77-81 (2003) (PMID: 12679418) (Abstract) described predictive value of serum thyroglobulin in thyroid cancer.

Mazzaferri et al., (J. Clin. Endocrinol. Metab. 88(4):1433-1441 (2003) (Abstract) describes thyroglobulin as a potential monitoring method for patients with thyroid carcinoma.

Whitley et al, (Clin. Lab. Med. 24(1):29-47 (2004) (Abstract) describes thyroglobulin as a serum marker for thyroid carcinoma.

Kuo et al (Clin. Chim. Acta. 294(1-2):157-168 (2000) (Abstract) describes serum matrix metalloproteinase-2 and –9 in HCF- and HBV-infected patients.

Koopman et al., (Cancer Epidemiol. Biomarkers Prev 13(3):487-491 (2004) (Abstract) describes osteopontin as a biomarker for pancreatic adenocarcinoma.

Pellegrini et al., (Cancer Immunol. Immunother. 49(7):388-394 (2000) (Abstract) describes measurement of soluble carcinoembryonic antigen and TIMP1 as markers for pre-invasive colorectal cancer.

Thus, we have identified numerous genes and/or proteins that are useful for developing reagents, devices and kits for detecting and evaluating gastric cancer. One or more markers of gastric can be used, either singly or in combination to provide a reliable molecular test for gastric cancer.

EXAMPLES

The examples described herein are for purposes of illustrating embodiments of the invention. Other embodiments, methods and types of analyses are within the scope of persons of ordinary skill in the molecular diagnostic arts and need not be described in detail hereon. Other embodiments within the scope of the art are considered to be part of this invention.

Example 1: Identification of Markers for Gastric Malignancy

Figure 2 depicts a table that shows results of studies using 38 markers for gastric malignancy selected using the above criteria. The Figure 2 includes the symbol for the gene ("symbol"), the MWG oligo number, the NCBI mRNA reference sequence number, the protein reference sequence number, the fold change between tumor and non-tumor gene expression, the fold change rank relative to other genes in the microarray analysis, the results of an original, unadjusted Student's t-test, the results of the Bonferroni-adjusted p value and the results of the 2-sample Wilcoxon test.

The median fold change (tumor: non malignant tissue) for these 34 genes ranged from 1.6 to 7 and the median change in fold change rank ranged from –16,995 to –25,783. The maximum possible change in fold change rank was -29,718. For each of the markers shown, the statistical significance of their specificity as cancer markers was found to be extremely high. The Bonferroni-adjusted p values were, in general, all below 10^{-6} or less, indicating that diagnosis using these markers is very highly associated with gastric cancer.

The three cystatins (CST1, CST2, and CST4) are highly homologous and represented by the same oligonucleotide on the microarray and unless otherwise stated, are referred to collectively as "CST1,2,4."

All proteins depicted in Figure 2 were predicted to have signal peptides using the SMART package (European Molecular Biology Laboratory). The signal peptides are known to target synthesized proteins to the extracellular compartment and can therefore be secreted into the interstitial fluid, from which they can have access to the blood. In fact, some proteins of this invention have been detected in serum.

Each of the genes depicted in Figure 2 exhibited a change in intensity rank greater than the two oligonucleotides on the array corresponding to CEA, the marker most frequently used in clinical practice to monitor gastric cancer progression.

Example 2: qPCR Analysis

More sensitive and accurate quantitation of gene expression was obtained for a subset of the genes shown in Figure 3 using qPCR. RNA from 46 tumor and 49 non-malignant samples was analyzed for 23 genes identified by the microarray analysis (Figure 2) and results are shown in Figure 3. Figure 3 includes the gene symbol, median fold change between cancer and normal tissue, and the % of tumor samples with expression levels greater than the 95th percentile of expression levels in non-malignant samples. 12 tumor samples and 9 normal samples were excluded from the analysis because of high (>75%) normal cell contamination, a high degree of necrosis (>40%), or poor hybridization signal on the microarrays. The median fold change (tumor tissues compared to the median non-malignant tissue expression) for these 23 genes ranged from 3 to 525 fold (Figure 3).

The level of expression of genes ASPN, CST1,2,4, LOXL2, TIMP1, SPP1, SFRP4, INHBA, THBS2 and SPARC was greater in tumors than the 95th percentile of the non-malignant range for ≥90% of cases (Figure 3). For the remainder of genes, the expression in tumors was greater than the 95th percentile in >50% of samples. Each tumor over-expressed at least seven genes greater than the 95th percentile indicating that combinations of markers will lead to comprehensive coverage of all gastric tumors.

Example 3: Validation of Array Data Using qPCR

Array data was validated using quantitative, real-time PCR (qPCR) on the tumor and non-malignant samples with probes for 24 genes. Of all 24 genes studied, 20 showed a strong correlation between the two techniques. Four of these analyses are show in Figures 4a – 4d, which depict graphs of the relative expression for the 4 selected cancer markers detected using array and qPCR methods. For each graph in Figure 4, the horizontal axis represents the array log2 fold change in gene expression, and the vertical axis represents the qPCR log2 fold change in gene expression. We found that there was a strong correlation between the two methods, as indicated by the co-variant relationship between the methods. The strong correlation indicates that both microarray fold change analysis and qPCR are suitable methods for detecting changes in the expression of gastric cancer marker genes and therefore can be used as an accurate, sensitive screening method. It can also be appreciated from Figures 4a – 4d that qPCR can be more sensitive at detecting changes in expression than are array methods. Thus, in situations in which early detection is especially desirable, qPCR may be especially useful.

Figures 5a – 5w depict histograms comparing frequency of observation of expression of each of a series of 23 genes (vertical axis) and the log2 fold change in expression for that gene (horizontal axis), for both normal tissue (open bars) and tumor tissues (black bars). We found surprisingly that for each of these 23 genes, there was substantial separation in the frequency distributions between normal and tumor tissue, as reflected by the low degree of overlap between the frequency distribution curves. For example, Figure 5b depicts the results for CST 1, 2, 4, for which there was only one normal sample observed to have an expression level in the tumor range. In other cases (e.g., Figure 5n; for PRS11) each frequency distribution curve was relatively narrow and there was a degree of overlap. However, even for this marker, the median log2 fold change showed a substantial separation of the amount of gene expression. In other cases, (e.g., Figure 5a; ASPN), although there was some overlap, there was a clear separation of the median log2 fold expression between normal and tumor samples.

Figure 6 depicts a histogram of the number of genes exhibiting a significantly increased expression ("over-expression") in tumor samples compared to normal samples (vertical axis) and the individual samples tested. In each case, the tumor sample exhibited multiple genes with elevated expression levels. The lowest number

of genes having increased expression was 7, found in sample E123. This finding indicates that, in situations in which multiple genes are over-expressed relative to normal tissue, the reliability of cancer detection can be very high, making diagnosis of cancer more certain. However, in some cases, elevation of expression of a single marker gene is sufficient to lead to the diagnosis of cancer.

Our previous comparison with the serum marker most frequently used currently for detection of gastric cancer, CEA, was based on difference in intensity rank of array data between tumors and normal samples. This comparison was verified using qPCR data for the markers and CEA.

Figures 7a-7c depict graphs of the relative log2 expression (compared to a reference RNA preparation) of markers in individual tumor samples and non-malignant samples compared to the expression of the gene for the tumor marker, CEA. CEA is the serum marker currently most used to monitor progression of gastric cancer. The zero point is defined to be the median normal expression for each marker. It can be seen that there is extensive overlap between the expression of the CEA gene (CEACAM5) in tumor samples and normal samples. This overlap is markedly less in the gastric cancer markers ASPN, CSPG2, CST1,2,4, IGFBP7, INHBA, LOXL2, LUM, SFRP4, SPARC, SPP1, THBS2, TIMP1, adlican, LEPRE1, and EFEMP2. For the other markers in Figures 7b-7c, ASAH1, SFRP2, GGH, MMP12, KLK10, TG, PRSS11 and TGFBI, the overlap between the tumor expression range and the non-malignant tissue expression range is greater than the overlap for the above markers, but still less than that of CEA, indicating that all of the herein described new markers are quantitatively better than CEA, and therefore can provide more reliable diagnosis.

To minimize effects of variable tissue handling, tumor:normal (non-malignant) fold changes were calculated using qPCR data from tumor and non-malignant tissue samples derived from the same patient. Such paired analysis corrects for differences in background levels of gene expression in different individuals and minimizes the effects of tissue handling on RNA quality. For example, if the resected stomach was at room temperature for an hour, the transcripts from the normal and tumor samples will be degraded to the same extent.

Figure 8 summarizes the T:N expression levels determined by qPCR for the markers, but used paired data (i.e., tumor and non-malignant samples) from the same individual. Figure 8 also includes expression data for six genes that were not included in Figure 3. The additionally studied genes are MMP2, CGR11, TGFB1, PCSK5,

SERPINB5, and SERPINH1. Identifying information and probes are shown in Figures 1 and 2. Figure 8 shows the median T:N fold change and the maximum T:N fold change for 29 gastric cancer markers in these 40 patients with "paired" samples. 27 of the 29 markers have a median T:N difference greater than or equal to the prior art marker, CEA. 29/29 of the markers have a higher percentage of paired samples in which the expression in the tumor sample exceeds the expression in the normal sample.

Figures 9a – 9d depict scatter dot plots of data from tumor and normal tissue from the same individuals. Each point represents the fold-change, within patient, in expression of the markers in tumor tissue relative to the expression in non-malignant tissue. All of the markers studied have better discrimination of tumor from non-tumor tissue than CEA. Three markers, CST1,2,4, ASPN and SFRP4 showed 100% discrimination between the paired tumor and normal samples. That is, for those markers, every tumor tissue had greater expression than did the corresponding non-tumor tissue from the same individual. In many other markers, for example, Adlican, CSPG2, EFEMP2, IGFBP7, INHBA, LOXL2, LUM, SERPINH1, SPARC, SPP1, TGFbI, THBS2 and TIMP1, each had only 2 or 3 individual points for which tumor tissue expression was less than that of the non-tumor tissue. Thus, for those markers, the likelihood that any one pair of tumor and non-tumor tissue would produce a false negative is relatively low (e.g., 3 of 40 or 7.5%; 2 of 40 or 5%, 1 of 40 or 2.5%). Thus, even if the other markers listed immediately above were used, use of multiple samples from an individual patient would produce reliable diagnostic information.

The gene sequences of these markers, and the location of the primers and probes used to detect them, are shown herein above.

To determine if over-expression of the marker genes is independent of the stage of the gastric tumors, the paired T:N log2 fold changes were plotted against the tumor stage (Figures 10a – 10ad). No stage dependency of expression on tumor stage was observed for 26 of the markers listed in Figure 8. These markers were similarly over-expressed in early stage as well as late stage tumors. However, KLK10 showed more consistent over-expression in stage 1 and stage 2 tumors, and PCSK5 and SERPINB5 showed more consistent over-expression in stage 4 tumors. KLK10, PCSK5 and SERPINB5 therefore can be used in determining the stage of gastric tumors.

In a similar analysis, paired T:N log2 fold changes were plotted against the Lauren classification of the tumor (either diffuse type or intestinal type). Figures 11a – 11ad show that each of the 29 GTMs discriminated between tumor and non-tumor tissue, regardless of whether the type of tumor was intestinal (I) or diffuse (D).

Example 4: Use of Multiple Markers

As described above, certain markers exhibit an ability to discriminate tumor from non-tumor tissue in 100% of the samples. Other markers, also described above, can be used in combination to achieve very high degrees of discrimination of tumor tissue from non-tumor tissue. Figure 12 depicts a 3-dimensional plot of the expression of 3 markers, SERPINH1, CST1,2,4 and INHBA, expressed as log2 T:N fold changes for a series of gastric tumor samples and non-malignant gastric samples. There is complete separation between the two groups of samples.

The reliability of successful discrimination of tumor and non-tumor samples using marker combinations is further illustrated by a statistical analysis summarized in Figure 13. This analysis compared the normal distributions of data generated using the qPCR gene expression from paired tumor and non-malignant samples, shows the effect of increasing the numbers of markers used to discriminate between tumor and non-malignant samples on test sensitivity (with a fixed specificity of 95%). Although few of the 29 markers (as shown in Figure 8) have a sensitivity of greater than 90, 95, or 99% when used alone in this analysis, the combination of two or three markers enabled high sensitivity to be reached with large numbers of combinations. For example, 50 combinations of three markers would discriminate between tumor and non-malignant samples with a sensitivity of ≥99% and specificity of ≥95%.

Example 5: Detection of Gastric Tumor Marker Proteins

In yet further embodiments, GTM proteins can be detected as a basis for diagnosis. In certain situations, the concentration of mRNA in a particular sample, such as a sample containing no cells, it may be difficult to use either microarray or qPCR methods to detect elevations in gene expression. Thus, in certain embodiments, detection of GTM proteins can be accomplished using antibodies directed against either the entire protein, a fragment of the protein (peptide) or the protein core. Methods for detecting and quantifying expression of proteins and peptides are known in the art and can include methods relying on specific antibodies raised against the

protein or peptide. Monoclonal antibodies and polyclonal antisera can be made using methods that are well known in the art and need not be described herein further.

To demonstrate that GTM proteins can be used to discriminate tumor from non-tumor tissue, commercial antibodies were obtained against SPARC (R&D Systems; cat # AF941), THBS2 (Santa Cruz Biotechnology Inc; cat # sc-7655), CSPG2 (Calbiochem; cat # 428060) and IGFBP7 (R&D Systems; cat # AF1334). An additional polyclonal antibody was raised in rabbits (Alpha Diagnostic International Inc; San Antonio) against the cystatin SN peptide sequence 50-66 (C) FAISEYNKATKDDYYRR. SEQ ID NO: 108.

These antibodies were used in either immunohistochemistry or Western analysis of tumor and non-malignant gastric tissue. Each of these markers showed strong tumor:normal differences at the protein level. This confirmed that the over-expression observed at the RNA level for these genes also occurred at the protein level.

Figure 14 shows a Western blot analyses of total protein extracted from two pairs of tumor and non-malignant tissues using antibodies against the proteins encoded by SPARC, CST1 (cystatin SN), IGFBP7 and THBS2. For each marker, the signal is significantly higher in the tumor samples than the non-malignant samples.

The antibody raised against cystatin SN detected three major bands, corresponding to molecular weights of approximately 34, 45 and 65kDa respectively. The lowest molecular weight band is shown in Figure 14. The protein species were larger than the control cystatin SN protein, suggesting that the protein produced by tumors has undergone post-translational modifications or multimerization. Regardless of the mechanism responsible for the differences in molecular weights of CST proteins, Figure 14 demonstrated that CST expression was low in the non-tumor tissue, but was easily observed in blots of tumor-derived proteins.

Figure 14 also showed that SPARC protein is expressed substantially to a greater degree in tumor tissue than in non-tumor tissue. The SPARC protein had gel mobility slower than the form of this protein that was detected in serum (Figure 15), also indicating the occurrence of different post-translational modifications in proteins produced by malignant gastric cells. Regardless of the mechanism(s) responsible for any such modification, the finding that SPARC is over-expressed in tumor tissue relative to non-malignant tissue indicates that SPARC is a useful protein marker.

Similarly, IGFBP7 and THBS2 show over-expression in tumor tissue relative to non-malignant tissue.

Immunohistochemical analysis of tumor and non-malignant tissue was carried out using antibodies against the proteins encoded by CSPG2 (versican) and CST1 (cystatin SN). Immunohistochemical analysis of tissue with antibodies against versican identified strong staining in the extracellular matrix of tumor tissue, but not non-malignant tissue. With the anti-cystatin SN antibodies, strong staining was observed in the area around the outside of the tumor cells. In non-malignant cells, the staining with this antibody was weaker, and observed only on the mucosal surface of the tissue and the lining of the gastric pits. This demonstrated that in non-malignant cells, cystatin SN protein is directed out of the cell onto the mucosal surface and not into the extracellular spaces. Therefore, not only is the cystatin SN protein being produced in higher amounts in tumor tissue than non-malignant tissue, but, unlike the protein produced by the non-malignant tissue, the tumor cystatin SN is in direct contact with the tissue vasculature. To extend these observations, cystatin SN was immunoprecipitated from the supernatant of the gastric cancer cell line, AGS with a monoclonal antibody (R&D Systems; cat # MAB1285) (Figure 16). Large amounts of cystatin SN were detected in the supernatant, confirming that this protein is produced by, and secreted from, gastric epithelial cells.

Example 6: Analysis of Tumor Markers in Serum

For a marker to be useful for rapid screening, it is desirable for the marker to be present in the serum in sufficient levels for detection. Certain proteins described in Figure 8 can be secreted into the blood at detectable levels from gastric cancers. One marker known to be secreted from gastric tumors into blood in detectable levels is TIMP1. However, if a protein is secreted or shed from any surface of a cell other than a mucosal surface, it will have contact with the interstitial fluid. From there, it can pass either directly into the blood supply through a capillary or via the lymph system. Thus, any shed GTM will be present in blood. Osteopontin, thyroglobulin, and members of the MMP and kallikrein families have previously been described to be elevated in the serum of patients with a range of epithelial cancers, but not gastric cancer. TIMP1 has, however, previously been observed to be elevated in the serum of gastric cancer patients. These findings suggest that the selection criteria for markers in this study, namely over-expression of secreted proteins in tumor tissue but not non-

malignant tissue, can be effectively used to detect markers in the serum, and thus can be of substantial use clinically, without the need for tissue or organ biopsies.

From Figure 15, it is apparent that the serum SPARC has a different molecular weight (depicted here in the Western blot) with the tumor SPARC having a lower molecular weight than the SPARC produced by blood cells. Thus, even though SPARC is produced by tumor and non-tumor blood cells, the presence of tumor SPARC can be determined using molecular size, such as determined using Western analysis, or with an antibody specific for the glycosylated protein produced by the tumor cells.

In another study, we detected cystatin SN in the supernatant of a gastric cancer cell line, AGS. Figure 16 depicts a Western analysis of media alone or a supernatant from AGS cells in culture. The right hand lane of Figure 16 shows a dense band corresponding to cystatin SN protein.

Thus, we conclude from Figure 10 that GTM of this invention are suitable for diagnosing gastric cancers at early, middle or late stages of progression of the disease.

Although certain marker proteins can be glycosylated, variations in the pattern of glycosylation can, in certain circumstances, lead to mis-detection of forms of GTMs that lack usual glycosylation patterns. Thus, in certain embodiments of this invention, GTM immunogens can include deglycosylated GTM or deglycosylated GTM fragments. Deglycosylation can be accomplished using one or more glycosidases known in the art. Alternatively, GTM cDNA can be expressed in glycosylation-deficient cell lines, such as prokaryotic cell lines, including E. coli, thereby producing non-glycosylated proteins or peptides. It can also be appreciated that the level and quality of glycosylation can be sensitive to the presence of essential precursors for sugar side-chains. Thus, in the absence of an essential sugar, "normal" glycosylation may not occur, but rather, shorter or missing side chain sugars may be found. Such "glycosylation variants" can be used as immunogens to produce antibodies specific for different types of marker genes.

Additionally, certain GTMs may form homo-or heterodimers or other types of multimeric forms. For example, inhibin beta A is a 47 kDa protein that can form homodimers of 97 kDa molecular weight (activin A) and 92 kDa heterodimers with the 45 kDa protein inhibin beta B (the heterodimers are known as activin AB). Thus, it can be appreciated that Western analysis or other type of assay that provides molecular weight need not be limited to only detection of a monomeric form of a

GTM. Rather, one can readily appreciate that any form of a GTM can be detected, regardless of the molecular weight. Thus, detection of a multimeric form of a GTM can be readily used to diagnose the presence of gastric cancer. Further, for those GTM that are selective for stage (1-4) or type of gastric tumor (diffuse or intestinal), detection of a multimeric form can provide suitable target for evaluating stage or type of gastric cancer.

Once an antibody or antiserum against a GTM is produced, such antibody preparations can be used for in a variety of ways. First, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) methods can be used to quantify GTM proteins or peptides. Immunodetection can be accomplished in tissue samples using immunohistochemistry. These methods are all known in the art and need not be described further herein.

Example 7: Vectors Containing GTM Oligonucleotides

Other embodiments of this invention include vectors useful for *in vitro* expression of marker genes or portions thereof ("marker peptides") or fragments of marker gene products. For example, vectors can be made having oligonucleotides for encoding GTMs therein. Many such vectors can be based on standard vectors known in the art. This invention also includes vectors that can be used to transfect a variety of cell lines to prepare GTM-producing cell lines, which can be used to produce desired quantities of GTMs for development of specific antibodies or other reagents for detection of GTMs or for standardizing developed assays for GTMs.

It is to be understood that to manufacture such vectors, an oligonucleotide containing the entire open reading frame or a portion of such an open reading frame encoding a portion of the protein to be expressed can be inserted into a vector containing a promoter region, one or more enhancer regions operably linked to the oligonucleotide sequence, with an initiation codon, an open reading frame, and a stop codon. Methods for producing expression vectors are known in the art and need not be repeated herein.

It can also be appreciated that one or more selectable markers can be inserted into an expression vector to permit the expansion of cell lines selected to contain the expression vector of interest. Moreover, one can also insert leader sequences known in the art, in frame, to direct secretion, internal storage or membrane insertion of the protein or protein fragment in the expressing cell.

Example 8: Cells Transfected with GTM-Containing Vectors

In still further embodiments, cells are provided that can express GTMs, GTM fragments or peptide markers. Both prokaryotic and eukaryotic cells can be so used. For example, E. coli (a prokaryotic cell) can be use to produce large quantities of GTMs lacking in mature glycosylation (if the particular GTM normally is glycosylated). COS cells, 293 cells and a variety of other eukaryotic cells can be used to produce GTMs that are glycosylated, or have proper folding and therefore, three-dimensional structure of the native form of the GTM protein. Methods for transfecting such cells are known in the art and need not be described further herein.

Example 9: Kits

Based on the discoveries of this invention, several types of test kits can be produced. First, kits can be made that have a detection device pre-loaded with a detection molecule (or "capture reagent"). In embodiments for detection of GTM mRNA, such devices can comprise a substrate (e.g., glass, silicon, quartz, metal, etc) on which oligonucleotides as capture reagents that hybridize with the mRNA to be detected. In some embodiments, direct detection of mRNA can be accomplished by hybridizing mRNA (labeled with cy3, cy5, radiolabel or other label) to the oligonucleotides on the substrate. In other embodiments, detection of mRNA can be accomplished by first making complementary DNA (cDNA) to the desired mRNA. Then, labeled cDNA can be hybridized to the oligonucleotides on the substrate and detected.

Regardless of the detection method employed, comparison of test GTM expression with a standard measure of expression is desirable. For example, RNA expression can be standardized to total cellular DNA, to expression of constitutively expressed RNAs (for example, ribosomal RNA) or to other relatively constant markers.

Antibodies can also be used in kits as capture reagents. In some embodiments, a substrate (e.g., a multiwell plate) can have a specific GTM capture reagent attached thereto. In some embodiments, a kit can have a blocking reagent included. Blocking reagents can be used to reduce non-specific binding. For example, non-specific oligonucleotide binding can be reduced using excess DNA from any convenient source that does not contain GTM oligonucleotides, such as salmon sperm DNA.

Non-specific antibody binding can be reduced using an excess of a blocking protein such as serum albumin. It can be appreciated that numerous methods for detecting oligonucleotides and proteins are known in the art, and any strategy that can specifically detect GTM associated molecules can be used and be considered within the scope of this invention.

In embodiments relying upon antibody detection, GTM proteins or peptides can be expressed on a per cell basis, or on the basis of total cellular, tissue, or fluid protein, fluid volume, tissue mass (weight). Additionally, GTM in serum can be expressed on the basis of a relatively high-abundance serum protein such as albumin.

In addition to a substrate, a test kit can comprise capture reagents (such as probes), washing solutions (e.g., SSC, other salts, buffers, detergents and the like), as well as detection moieties (e.g., cy3, cy5, radiolabels, and the like). Kits can also include instructions for use and a package.

Although this invention is described with reference to specific embodiments thereof, it can be appreciated that other embodiments involving the use of the disclosed markers can be used without departing from the scope of this invention.

INDUSTRIAL APPLICABILITY

Methods for detecting GTM family members include detection of nucleic acids using microarray and/or real time PCR methods and detection of proteins and peptides. The compositions and methods of this invention are useful in the manufacture of diagnostic devices and kits, diagnosis of disease, evaluating efficacy of therapy, and for producing reagents suitable for measuring expression of GTM family members in biological samples.

We Claim:

1. A method for detecting gastric cancer, comprising:
   (a) providing a biological sample; and
   (b) detecting over-expression of a GTM family member in said sample.

2. The method of claim 1, wherein said GTM family member is selected from the group consisting of carboxypeptidase N, polypeptide 2, 83 kDa chain (CPN2), matrix metalloproteinase 12 (MMP12), inhibin ("INHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline-enriched proteoglycan ("LEPRE1"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein 10 (KLK10), tissue inhibitor of metalloproteinase 1 ("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor, β-induced ("TGFBI"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAH1"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlican, cystatin SA ("CST2"), cystatin SN ("CST1"), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta1 ("TGFB1"), serine or cysteine proteinase inhibitor clade H ("SERPINH1"), serine or cysteine proteinase inhibitor clade B ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5") and hyaluronan glycoprotein link protein 4 ("HAPLN4").

3. The method of claims 1 or 2, wherein said step of detecting is carried out by detecting over-expression of GTM mRNA.

4. The method of claims 1 or 2, wherein said step of detecting is carried out by detecting over-expression of GMT cDNA.

5. The method of claim 4, wherein said step of detecting is carried out using an oligonucleotide complementary to at least a portion of said GMT cDNA.

6. The method of claim 4, wherein said step of detecting is carried out using qPCR method using a forward primer and a reverse primer.

7. The method of claims 1 or 2, wherein said step of detecting is carried out by detecting over expression of a GTM protein.

8. The method of claims 1 or 2, wherein said step of detecting is carried out by detecting over expression of a GTM peptide.

9. The method of claims 7 or 8, wherein said step of detecting is carried out using an antibody directed against said GMT.

10. The method of any of claims 7-9, wherein said step of detecting is carried out using a sandwich-type immunoassay method.

11. The method of any of claims 7-10, wherein said antibody is a monoclonal antibody.

12. The method of any of claims 7-10, wherein said antibody is a polyclonal antiserum.

13. A device for detecting a GTM, comprising:

a substrate having a GTM capture reagent thereon; and

a detector associated with said substrate, said detector capable of detecting a GTM associated with said capture reagent.

14. The device of claim 13, wherein said GTM capture reagent is an oligonucleotide.

15. The device of claim 13, wherein said GTM capture reagent is an antibody specific for either a GTM oligonucleotide, a GTM protein or a GTM peptide.

16. A kit for detecting cancer, comprising:
a substrate having a GTM capture reagent thereon;
a means for visualizing a complex of said GMT capture agent and a GMT;
reagents; and
instructions for use.

17. The kit of claim 16, wherein said GTM capture reagent is a GTM-specific oligonucleotide.

18. The kit of claim 16, wherein said GTM capture reagent is a GTM-specific antibody selective for a GTM ologinucleotide, a GTM protein or a GTM peptide.

19. A method for detecting gastric cancer, comprising the steps of:
providing a test sample from a patient suspected of having gastric cancer;
measuring the presence of a GTM protein in said test sample; and
comparing the amount of GTM present in said test sample with a value obtained from a control sample from a subject not having gastric cancer.

20. A method for screening for gastric cancer, comprising the steps of:
providing a test sample from a test subject;
measuring the presence of a GTM in said test sample; and
comparing the amount of GTM present in said test sample with a value obtained from a control sample from a subject not having gastric cancer.

21. The method of claim 19, wherein said GTM is a GTM protein or peptide.

22. The method of claim 19, wherein said GTM is an oligonucleotide specific for a GTM.

23. The method of claim 22, wherein said oligonucleotide is DNA.

24. The method of claim 22, wherein said oligonucleotide is RNA.

25. The method of any of claims 18 – 24, wherein said step of measuring uses an ELISA assay.

26. The method of any of claims 19-21, wherein said test sample is obtained from plasma.

27. The method of any of claims 19-21, wherein said test sample is obtained from tissue, urine, gastric fluid, serum and stool.

name	symbol	Applied Biosystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	probe	Seq ID No.
asporin (irr class 1)	ASPN		AAATACAAAAGGACACATTCAAAGGA	1	TGCTTCTGCAATTCTGATATGGA	23	TTGGAAATGAGTGCAAACCCTCTTGATAATAATG	45
chondroitin sulfate proteoglycan 2 (versican)	CSPG2		GCCAGTGGAATGATGTTCCC	2	TCTTGGCATTTTCTACAACAGGG	24	AGGAACAGTTGCTTGCGGCCAGC	46
cystatins SN, SA & S	CST1, 2, 4		AGTCCCAGCCCAACTTGGA	3	GGGAACTTCGTAGATCTGGAAAGA	25	AGCCAGAACTGCAGAAGAAACAGTTGTGC	47
gamma-glutamyl hydrolase	GGH		GTGGCAATGCCGCTGAA	4	TGACAGCAACAACTCAGTAGGAAAA	26	TTCACTGGAGGTCAATTGCACAGCAGAAT	48
insulin-like growth factor binding protein 7	IGFBP7		CAGGTCAGCAAGGGCACC	5	TCACAGCTCAAGTACACCTGGG	27	AGCAAGGTCCTTCCATAGTGACGCCC	49
kallikrein 10	KLK10		ACAACATGATATGTGCTGGACTGG	6	GAGAGGATGCCTTGGAGGGT	28	CTTGCCAGAGTGACTCTGGAGGCCC	50
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1		CTTGAGTACAACGCTGACCTCTTC	7	CCGTGACACAGTTCTGCTTACAG	29	CCATCACAGATCATTACATCCAGGTCCTCA	51
lumican	LUM		GATTCTTGTCCATAGTGCATCTGC	8	CCAATCAATGCCAGGAAGAGA	30	TAAGGATTCAAACCATTTGCCAAAAATGAGTCTAAG	52
lysyl oxidase-like 2	LOXL2		AGGCCAGCTTCTGCTTGGA	9	CCCTGATCGCCGAGTTG	31	CGTAATTCTTCTGGATGTCTCCTTCACATTCTG	53
matrix metalloproteinase 12	MMP12		GCCTCTCTGCTGATGACATACGT	10	AGTGACAGCATCAAAACTCAAATTG	32	TCAGTCCCTGTATGGAGACCCAAAAGAGAA	54
metalloproteinase inhibitor 1	TIMP1		CCAGACCACCTTATACCAGCG	11	GGACCTGTGGAAGTATCCGC	33	CAAGATGACCAAGATGTATAAAGGGTTCCAAGC	55
n-acylsphingosine amidohydrolase	ASAH1		CGCAGAACGCCTGCAAA	12	ACAGGACATCATACATGGTTTCAAA	34	TGTCTGAACCGCACCAGCCAAGAGAATA	56
secreted frizzled-related protein 2	SFRP2		CGCTAGCAGCGACCACCT	13	TTTTGCAGGCTTCACATACCTTT	35	CTGCCAGCCACCGAGGAAGCTC	57
secreted protein, acidic, cysteine rich	SPARC		TCTTCCCTGTACACTGGCAGTTC	14	GAAAAAGCGGGTGGTGCA	36	TGGACCAGCACCCCATTGACGG	58
serine protease 11 (IGF binding)	PRSS11		TCGGGAGGCCCGTTAGTAA	15	AAGGAGATTCCAGCTGTCACTTTC	37	AGTGTTAATTCCAATCACTTCACCGTCCAGG	59
thrombospondin 2	THBS2		TGGAAGGACTACACGGCCTATAG	16	TAGGTTTGGTCATAGATAGGTCCTGAGT	38	AGGCCCAAGACCGGCTACATCAGAGTC	60
thyroglobulin	TG		GACGGTTCCTCGCAGTTCAA	17	TGTAAACCGCTCCACTTCACAT	39	TCTGGCAGATTCCGATGCCCCACAA	61
human cell growth regulator with EF hand domain 1	CGR11		CTGCCCACCCCTTCCA	18	TTCTGTCCTTCCTAGTCCCTTTAGG	40	CCAGGCCAGGAGCAGCTCGG	62
human serine or cysteine proteinase inhibitor clade B	SERPINB5		TCCACGCATTTTCCAGGATAA	19	AAGCCGAATTTGCTAGTTGCA	41	TGACTCCAGGCCCGCAATGGA	63
transforming growth factor β1	TGFB1		GGTCCATGTCATCACCAATGTT	20	TCTGCAAGTTCATCCCCTCTTT	42	CAGCCTCCAGCCAACAGACCTCAGG	64
human proprotein convertase subtilisin/kexin type 5	PCSK5		AAAAATCTTTGCCGGAAATGC	21	AGTCCTGGCCGTTGAAATACC	43	ACAGAATGTAGGGATGGGTTAAGCCTGCA	65
matrix metalloproteinase 2	MMP2		TTGATGGCATCGCTCAGATC	22	TGTCACGTGGCGTCACAGT	44	TTCAAGGACCGGTTCATTTGGCG	66
human serine or cysteine proteinase inhibitor clade H	SERPINH1	Hs00241844_m1						
adlican	-	Hs00377849_m1						
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	Hs00213545_m1						
secreted frizzled-related protein 4	SFRP4	Hs00180066_m1						
inhibin beta A chain	INHBA	Hs00170103_m1						
osteopontin	SPP1	Hs00167093_m1						
transforming growth factor B-induced	TGFBI	Hs00165908_m1						
			Figure 1					

Microarray - Identification of Markers for Gastric Malignancy

name	symbol	MWG oligo #	NCBI mRNA ref sequence	protein ref sequence	fold change	fold change rank	original t-test	Bonferroni-adjusted p value	2 sample Wilcoxon test
adlican	-	C:0531	NM_015419	NP_056234	1.8	-17818	1.0E-28	3.04E-24	0.0E+00
asporin (lrr class 1)	ASPN	A:07749	NM_017680	NP_060150	2.6	-22292	6.4E-23	1.9E-18	0.0E+00
carboxypeptidase N	CPN2	B:4922	-	P22792	2.7	-22367.5	2.3E-42	7.0E-38	0.0E+00
cell growth regulatory factor with EF-hand domain	CGR11	A:07876	NM_006569	NP_006560	3.0	-21188.5	4.33E-42	1.3E-37	0.0E+00
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	A:10008	NM_004385	NP_004376	2.3	-21606.5	2.23E-33	6.65E-29	0.00E+00
cystatin SN	CST1	A:06089	NM_001898	NP_001889	2.1	-17475	1.3E-18	3.8E-14	0.0E+00
cystatin SA	CST2	A:06089	NM_001322	NP_001313	2.1	-17475	1.3E-18	3.8E-14	0.0E+00
cystatin S	CST4	A:06089	NM_001899	NP_001890	2.1	-17475	1.3E-18	3.8E-14	0.0E+00
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	A:09072	NM_016938	NP_058634	2.4	-22761	2.0E-35	5.9E-31	0.0E+00
gamma-glutamyl hydrolase	GGH	A:03601	NM_003878	NP_003869	1.6	-18092	1.6E-07	4.8E-03	5.7E-11
inhibin beta A chain	INHBA	A:02189	NM_002192	NP_002183	2.1	-21247	1.4E-30	4.3E-26	0.0E+00
insulin-like growth factor binding protein 7	IGFBP7	A:03385	NM_001553	NP_001544	3.0	-25854	5.4E-31	1.6E-26	0.0E+00
kallikrein 10	KLK10	A:07907	NM_002776	NP_002767	2.3	-17986.5	5.0E-10	1.5E-05	4.9E-06
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1	A:04646	NM_022356	NP_071751	1.7	-18019	8.2E-14	2.4E-09	1.1E-12
lumican	LUM	A:09199	NM_002345	NP_002336	2.9	-24927	4.2E-24	1.3E-19	0.0E+00
lysyl oxidase-like 2	LOXL2	A:06085	NM_002318	NP_002309	1.6	-16994.5	5.9E-10	1.7E-05	7.9E-10
matrix metalloproteinase 2	MMP2	A:06749	NM_004530	P08253	1.8	-18710	1.2E-11	3.6E-07	1.5E-10
matrix metalloproteinase 12	MMP12	A:01762	NM_002426	NP_002417	2.1	-20209.5	2.2E-12	6.6E-08	4.9E-11
metalloproteinase inhibitor 1	TIMP1	A:08048	NM_003254	NP_003245	3.2	-24177	7.5E-38	2.3E-33	0.0E+00
n-acylsphingosine amidohydrolase	ASAH1	A:10030	NM_004315	NP_004306	1.7	-19636.5	9.6E-16	2.9E-11	0.0E+00
olfactomedin	OLFM1	B:3555	NM_014279	NP_055094	3.9	-25782.5	6.5E-46	1.9E-41	0.0E+00
osteopontin	SPP1	A:09441	NM_000582	NP_000573	7.0	-26668	4.0E-32	1.2E-27	0.0E+00
human proprotein convertase subtilisin/kexin type 5	PCSK5	A:00704	NM_006200	Q92824	1.7	-18736	2.0E-11	6.0E-07	7.3E-11
group xiii secreted phospholipase a2	PLA2G12b	B:1811	NM_032562	NP_115951	3.0	-23212	7.92E-39	2.36E-34	0.00E+00
secreted frizzled-related protein 2	SFRP2	B:1634	XM_050625	XP_050625	2.1	-19217	2.7E-10	8.1E-06	4.1E-08
secreted frizzled-related protein 4	SFRP4	A:07398	NM_003014	NP_003005	3.0	-22153	6.0E-24	1.8E-19	0.0E+00
serine (or cysteine) proteinase inhibitor clade H	SERPINH1	A:08615	NM_001235	NP_001226	1.9	-20252	2.8E-34	8.2E-30	0.0E+00
human serine or cysteine proteinase inhibitor clade B	SERPINB5	A:10485	NM_002639	P36952	1.5	-17026	4.6E-06	1.4E-01	5.6E-06
serine protease 11 (IGF binding)	PRSS11	B:1274	NM_002775	NP_002766	1.6	-17184.5	9.3E-18	2.8E-13	0.0E+00
secreted protein, acidic, cysteine rich	SPARC	A:08092	NM_003118	NP_003109	2.5	-22947.5	1.5E-44	4.6E-40	0.0E+00
spondin 2	SPON2	B:2543	NM_012445	NP_036577	2.4	-20390.5	2.9E-31	8.5E-27	0.0E+00
stannin	SNN	A:09316	NM_003498	NP_003489	2.1	-20162.5	3.25E-24	9.71E-20	0.00E+00
thrombospondin 2	THBS2	B:9017	NM_003247	NP_003238	2.6	-22095	5.8E-29	1.7E-24	0.0E+00
thrombospondin repeat containing 1	TSRC1	B:7686	NM_019032	NP_061905	2.6	-22608	1.38E-45	4.1E-41	0.0E+00
thyroglobulin	TG	B:5402	NM_003235	NP_003226	2.4	-23644	4.39E-36	1.3E-31	0.0E+00
transforming growth factor β-induced	TGFBI	A:08124	NM_000358	NP_000349	2.5	-23339.5	1.96E-24	9.71E-20	0.0E+00
transforming growth factor β1	TGFB1	A:07050	NM_000660	P01137	1.6	-17214	2.30E-18	6.86E-14	0.0E+00
hyaluronan and proteoglycan link protein 4	HAPLN4	C:6300	NM_023002	NP_075378	3.4	-23516.5	7.32E-44	2.2E-39	0.0E+00

Figure 2

Quantitative RT-PCR - Quantification of Expression of Selected Gastric Cancer Candidate Genes					
name	symbol	median T:N fold change	Maximum T:N fold change	% T >95th percentile [1]	
adlican		5	37	74	
asporin (irr class 1)	ASPN	12	73	91	
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	6	24	78	
cystatins SN, SA & S	CST1, 2, 4	525	25532	100	
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	15	56	
gamma-glutamyl hydrolase	GGH	5	36	67	
inhibin beta A chain	INHBA	34	357	98	
insulin-like growth factor binding protein 7	IGFBP7	4	19	80	
kallikrein 10	KLK10	5	633	70	
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1	4	17	72	
lumican	LUM	5	47	80	
lysyl oxidase-like 2	LOXL2	6	26	93	
matrix metalloproteinase 12	MMP12	9	586	67	
metalloproteinase inhibitor 1	TIMP1	8	19	91	
n-acylsphingosine amidohydrolase	ASAH1	3	7	63	
osteopontin	SPP1	40	481	96	
secreted frizzled-related protein 2	SFRP2	5	85	63	
secreted frizzled-related protein 4	SFRP4	56	600	100	
secreted protein, acidic, cysteine rich	SPARC	9	56	93	
serine protease 11 (IGF binding)	PRSS11	4	25	54	
thrombospondin 2	THBS2	25	239	91	
thyroglobulin	TG	5	153	54	
transforming growth factor B-induced	TGFBI	7	204	82	
[1] percentage of tumors with expression levels greater than the 95th percentile of non-malignant samples.					

Figure 3

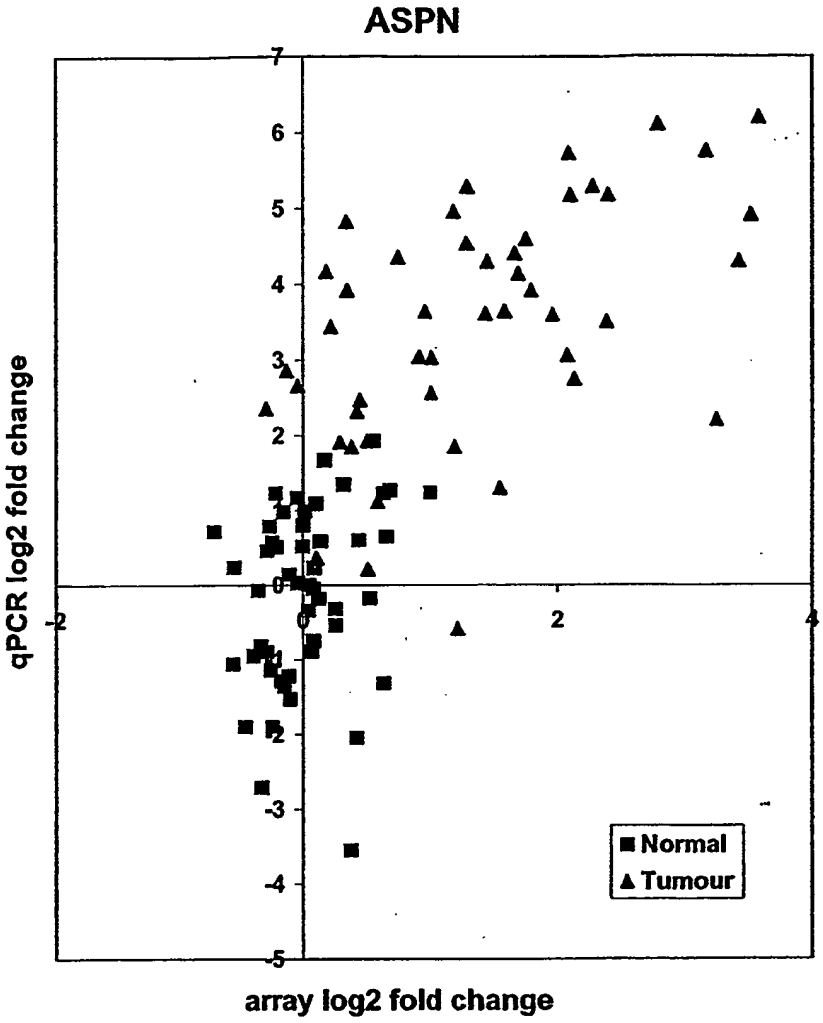

ASPN
qPCR log2 fold change
array log2 fold change
Normal
Tumour

Figure 4(a)

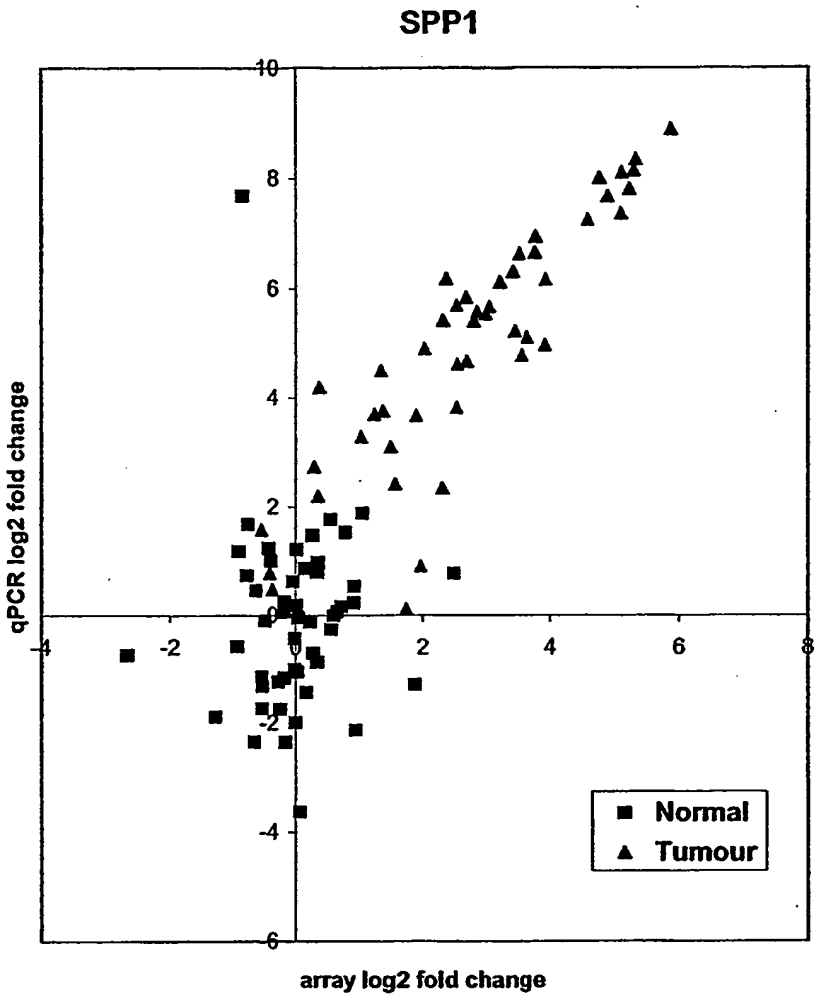

SPP1
qPCR log2 fold change
array log2 fold change
10
8
6
4
2
0
-2
-4
-6
-4
-2
2
4
6
8
Normal
Tumour

Figure 4(b)

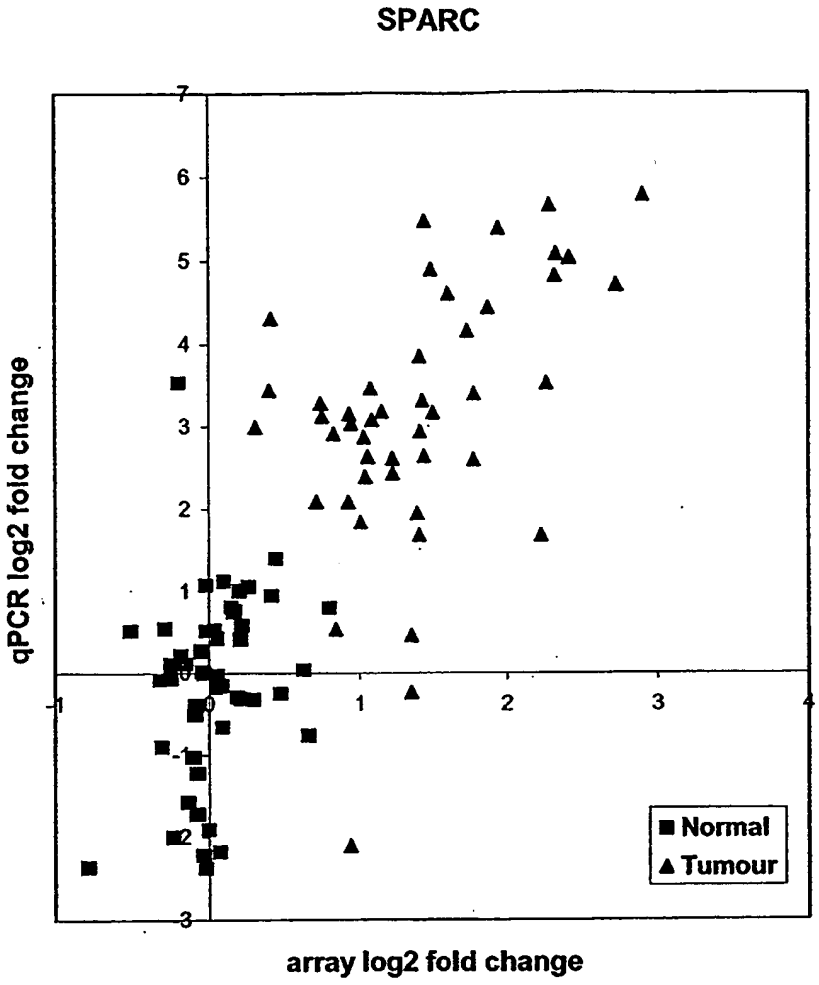

SPARC
qPCR log2 fold change
array log2 fold change
Normal
Tumour

Figure 4(c)

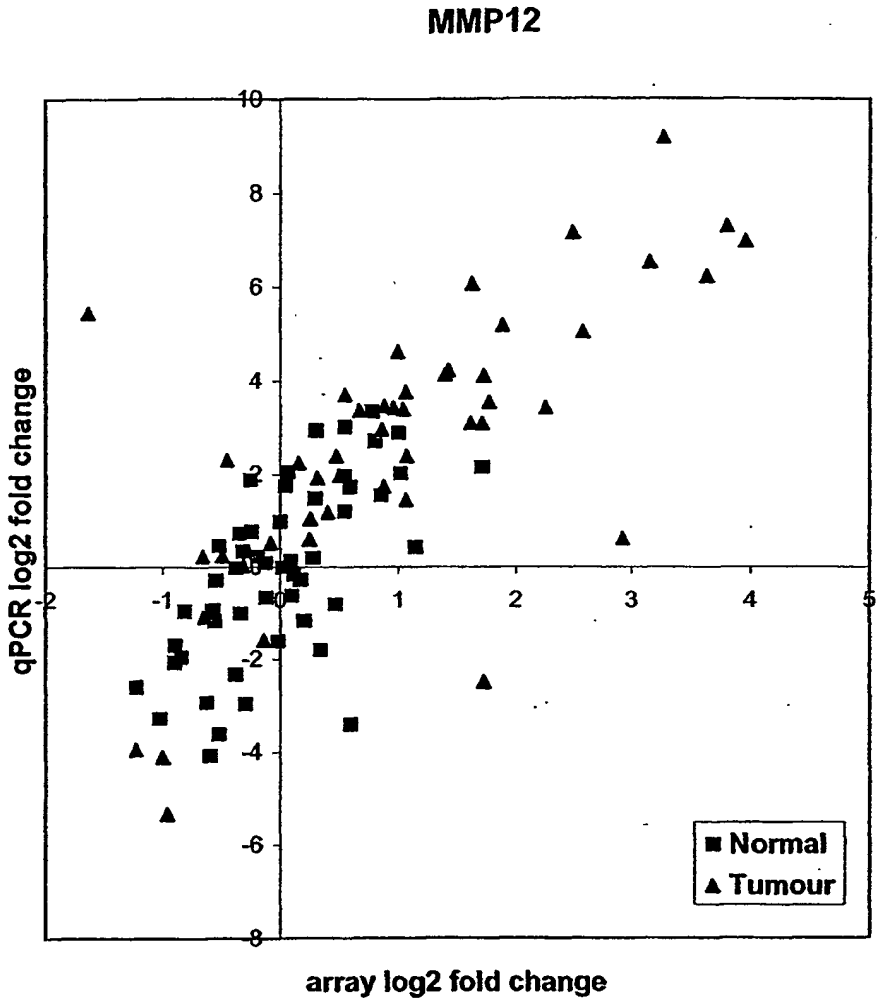

MMP12
qPCR log2 fold change
array log2 fold change
10
8
6
4
2
0
-2
-4
-6
-8
-2
-1
0
1
2
3
4
5
Normal
Tumour

Figure 4(d)

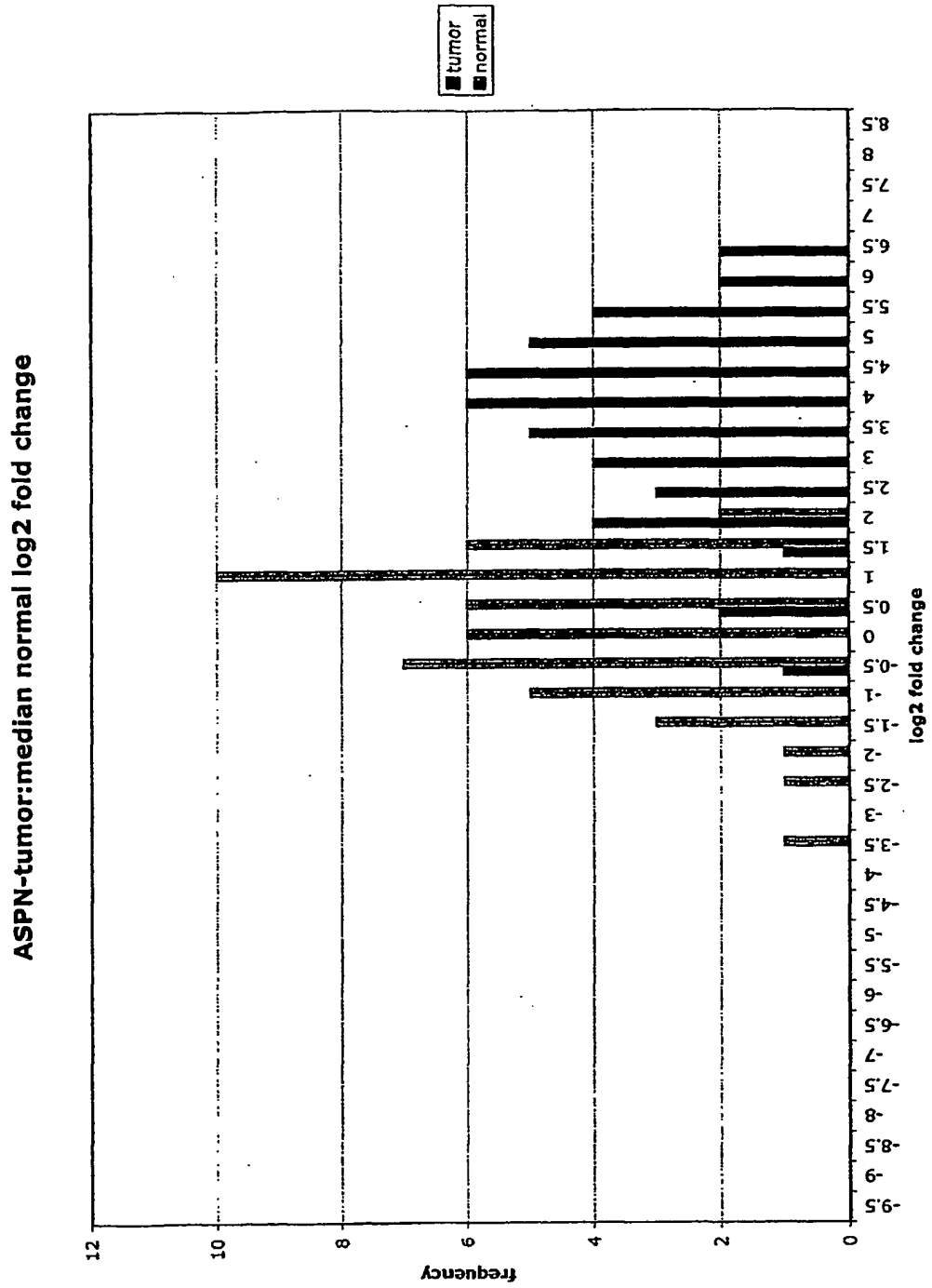

ASPN-tumor:median normal log2 fold change
tumor
normal
frequency
log2 fold change
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(a)

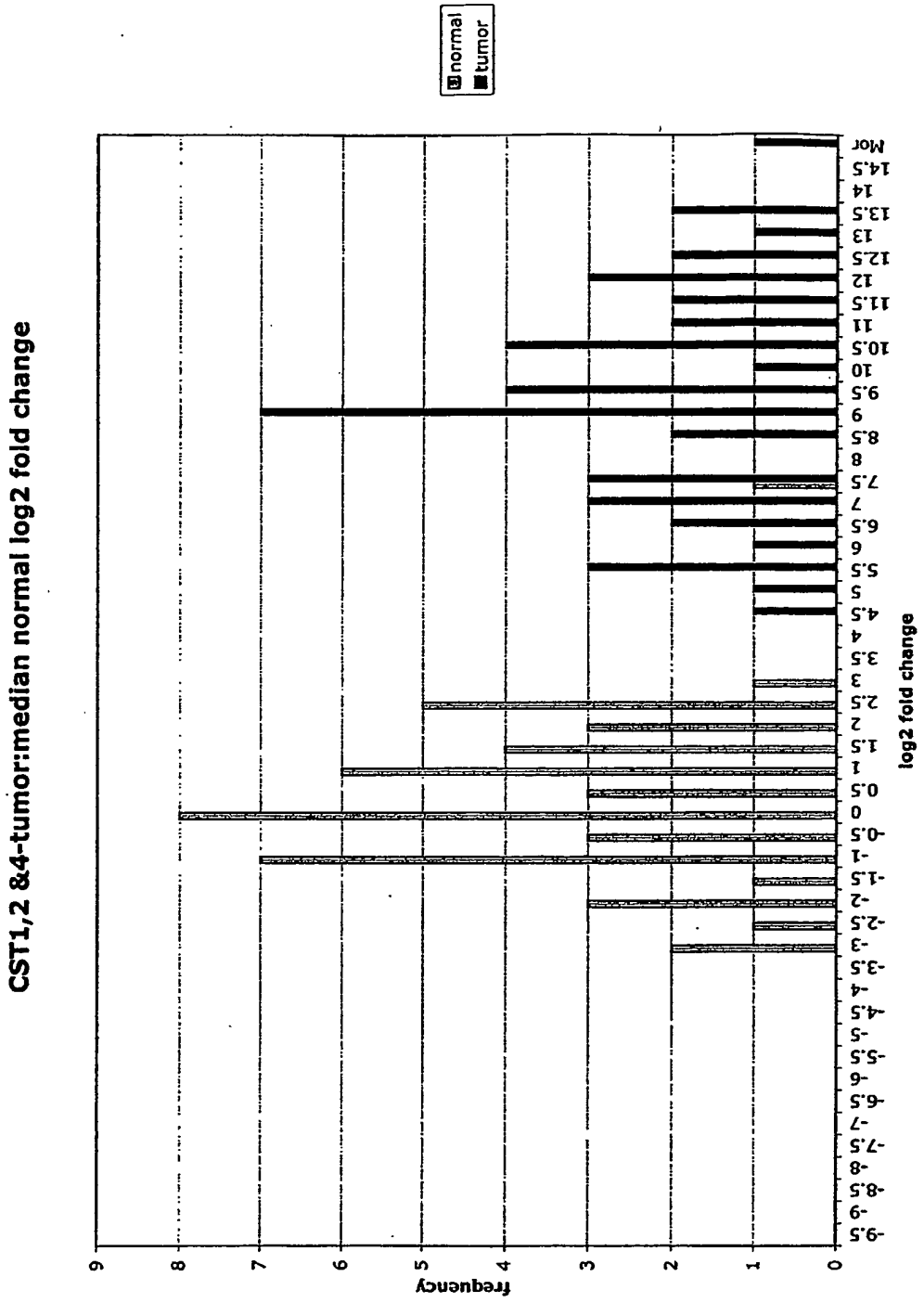

CST1,2 &4-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
0
1
2
3
4
5
6
7
8
9
-9.5
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10
10.5
11
11.5
12
12.5
13
13.5
14
14.5
More

Figure 5(b)

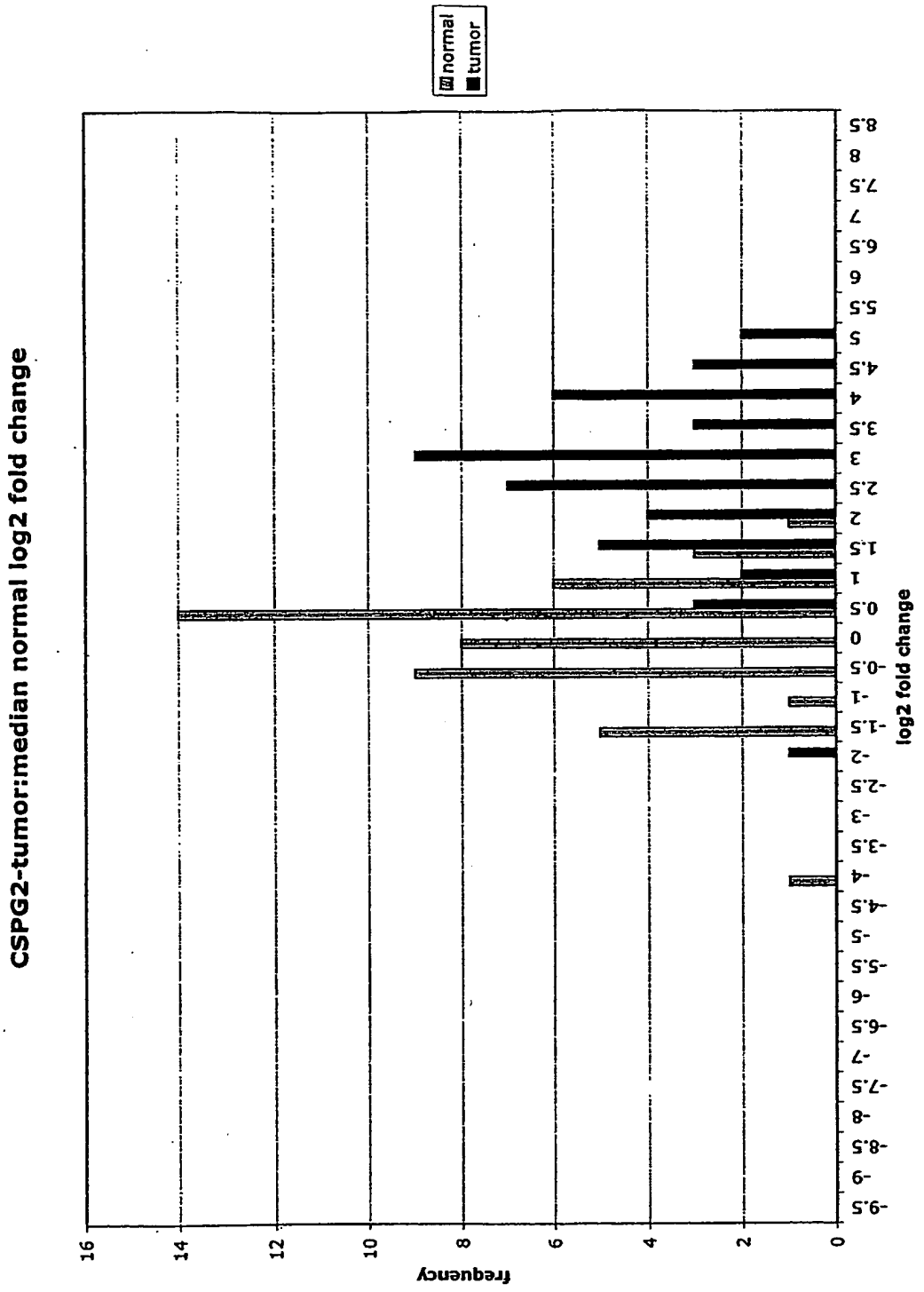

CSPG2-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
16
14
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(c)

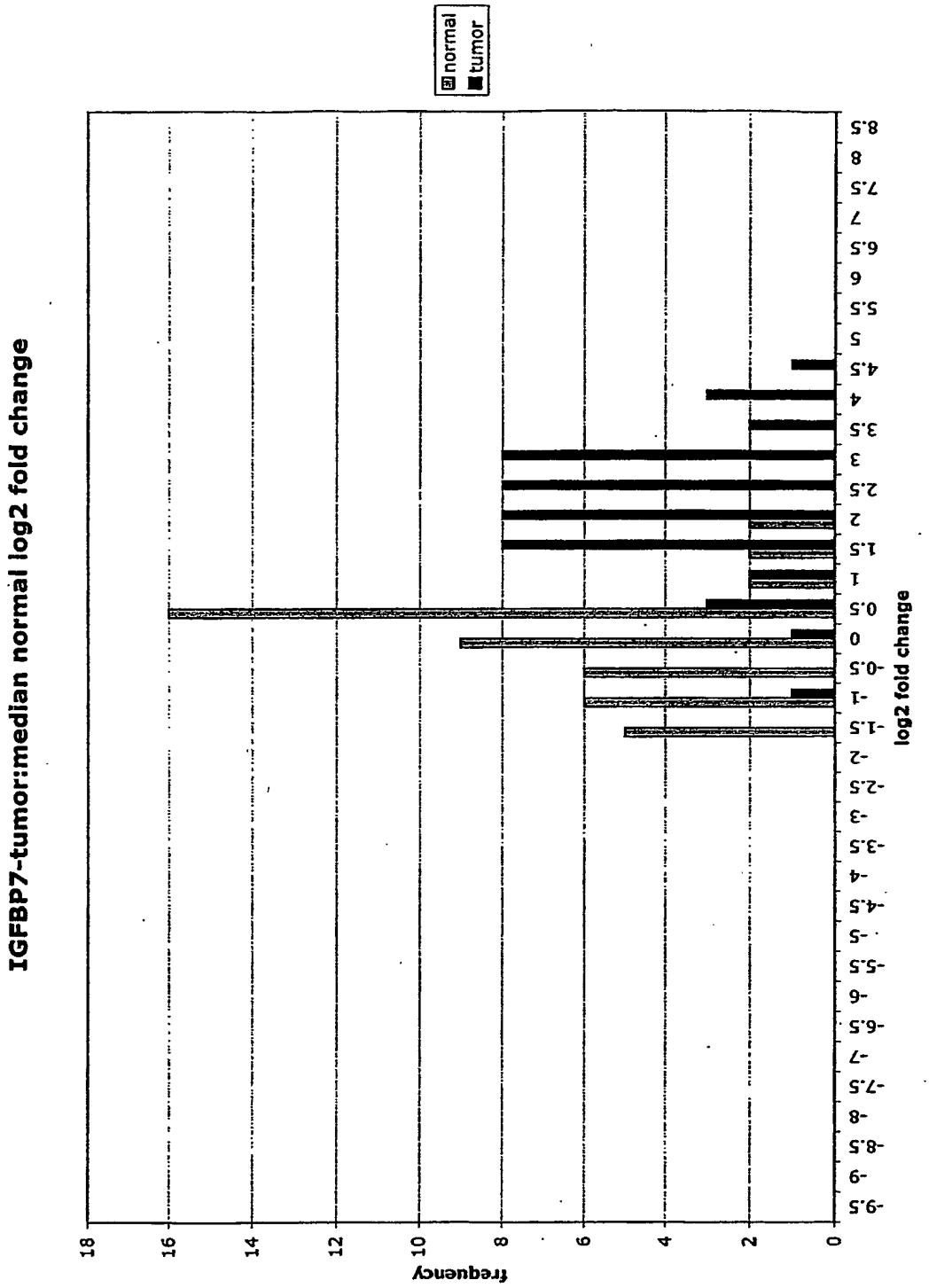

IGFBP7-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
18
16
14
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(d)

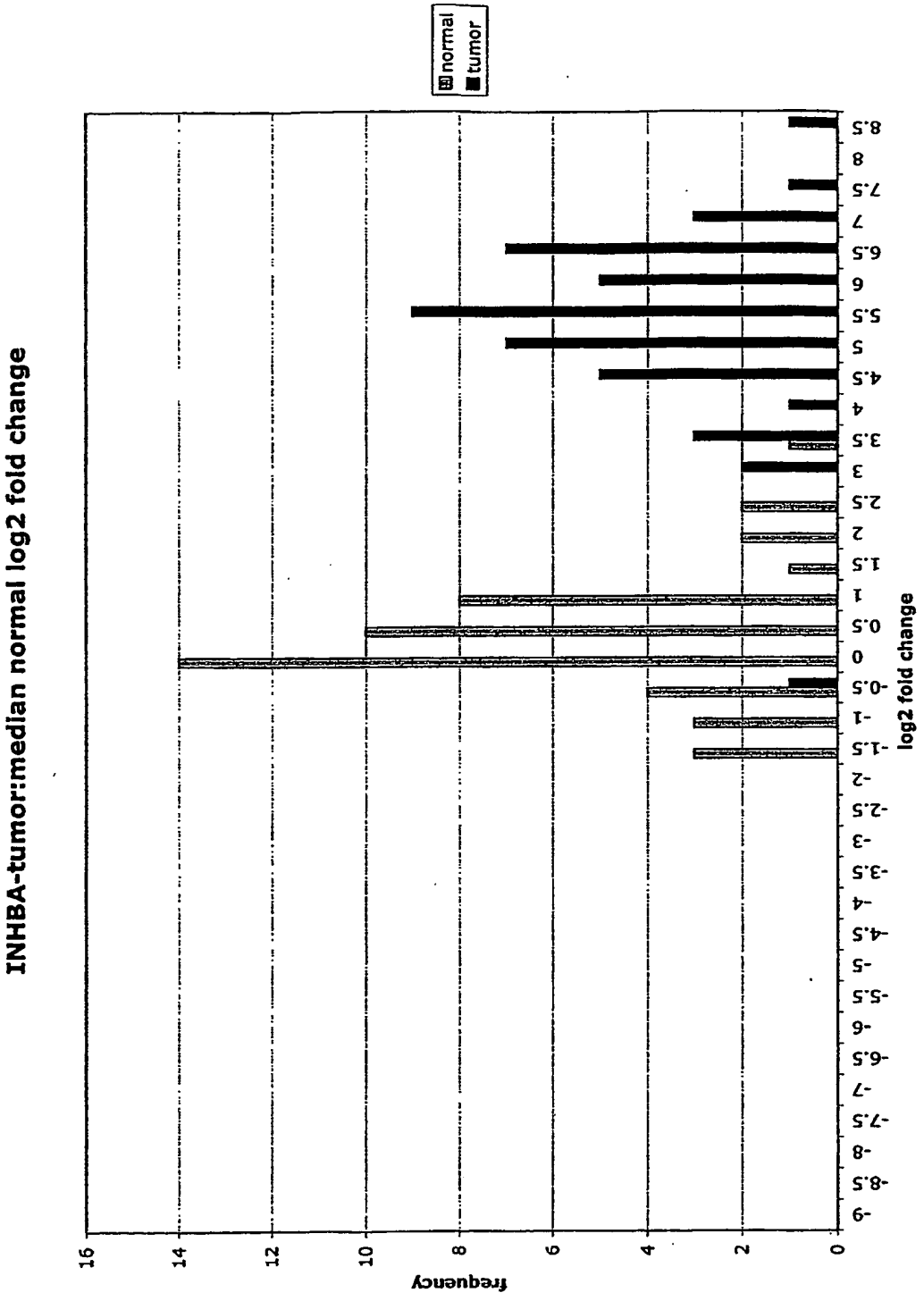

INHBA-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
16
14
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9

Figure 5(e)

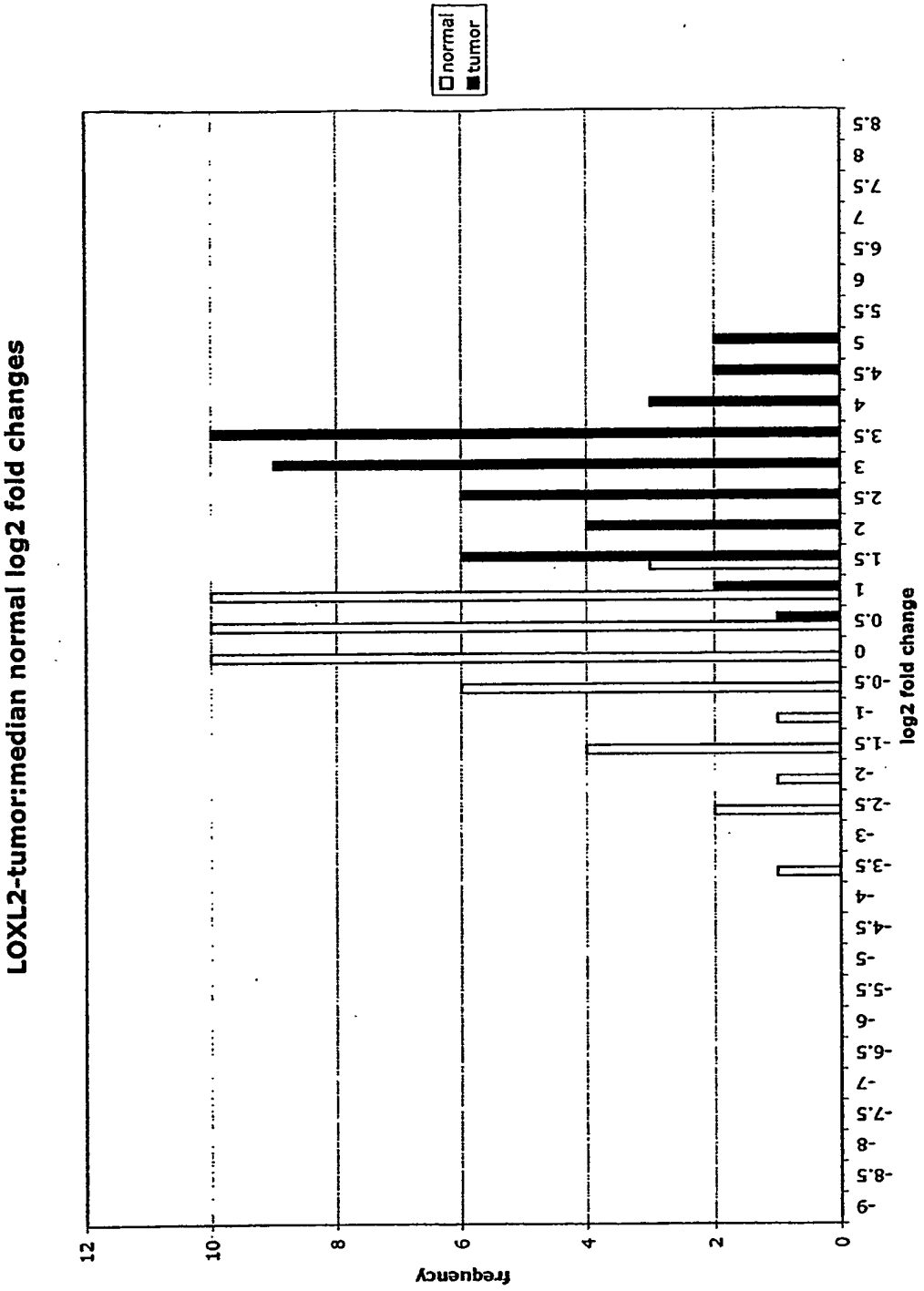

LOXL2-tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold change
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9

Figure 5(f)

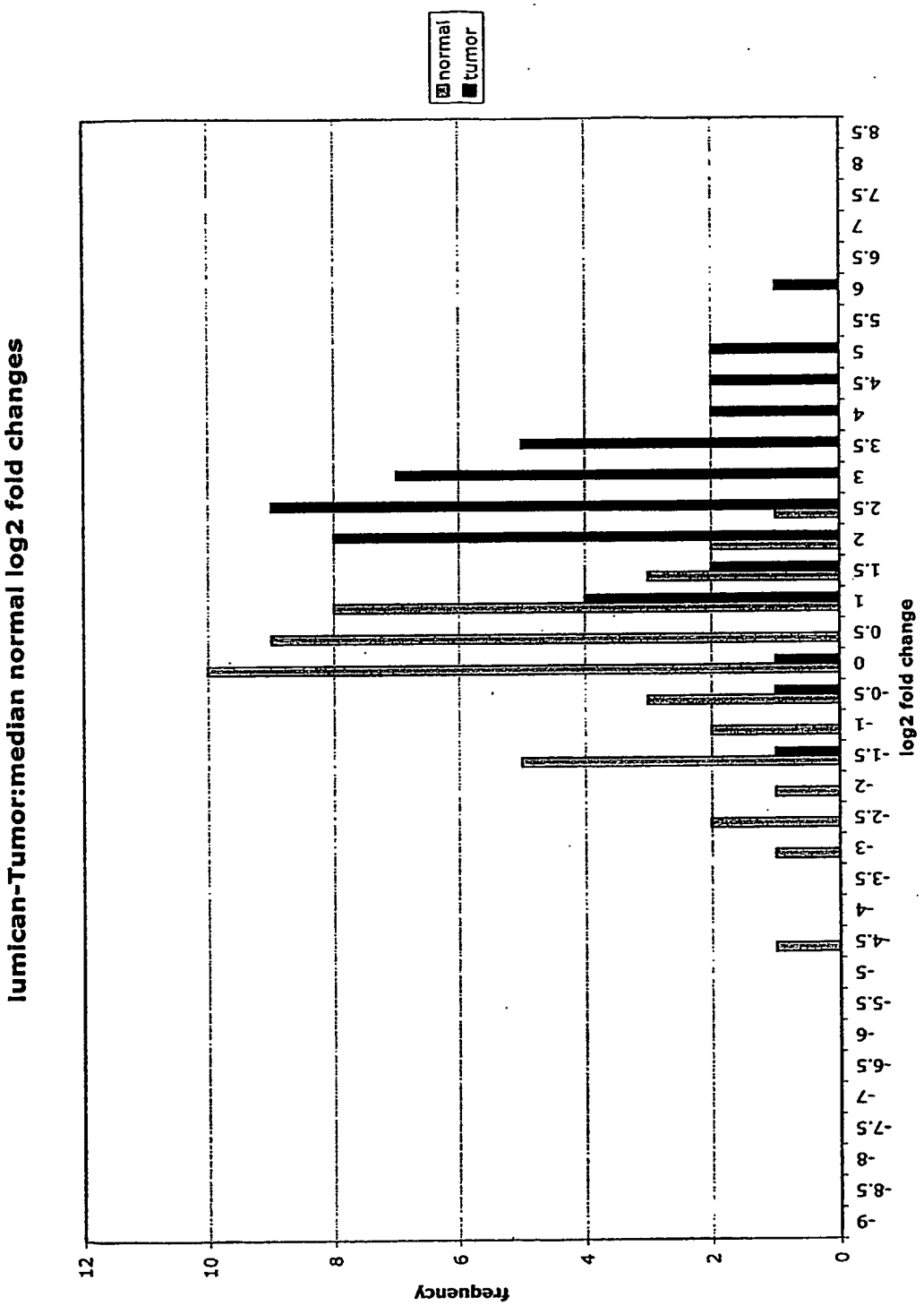

lumican-Tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold change
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9

Figure 5(g)

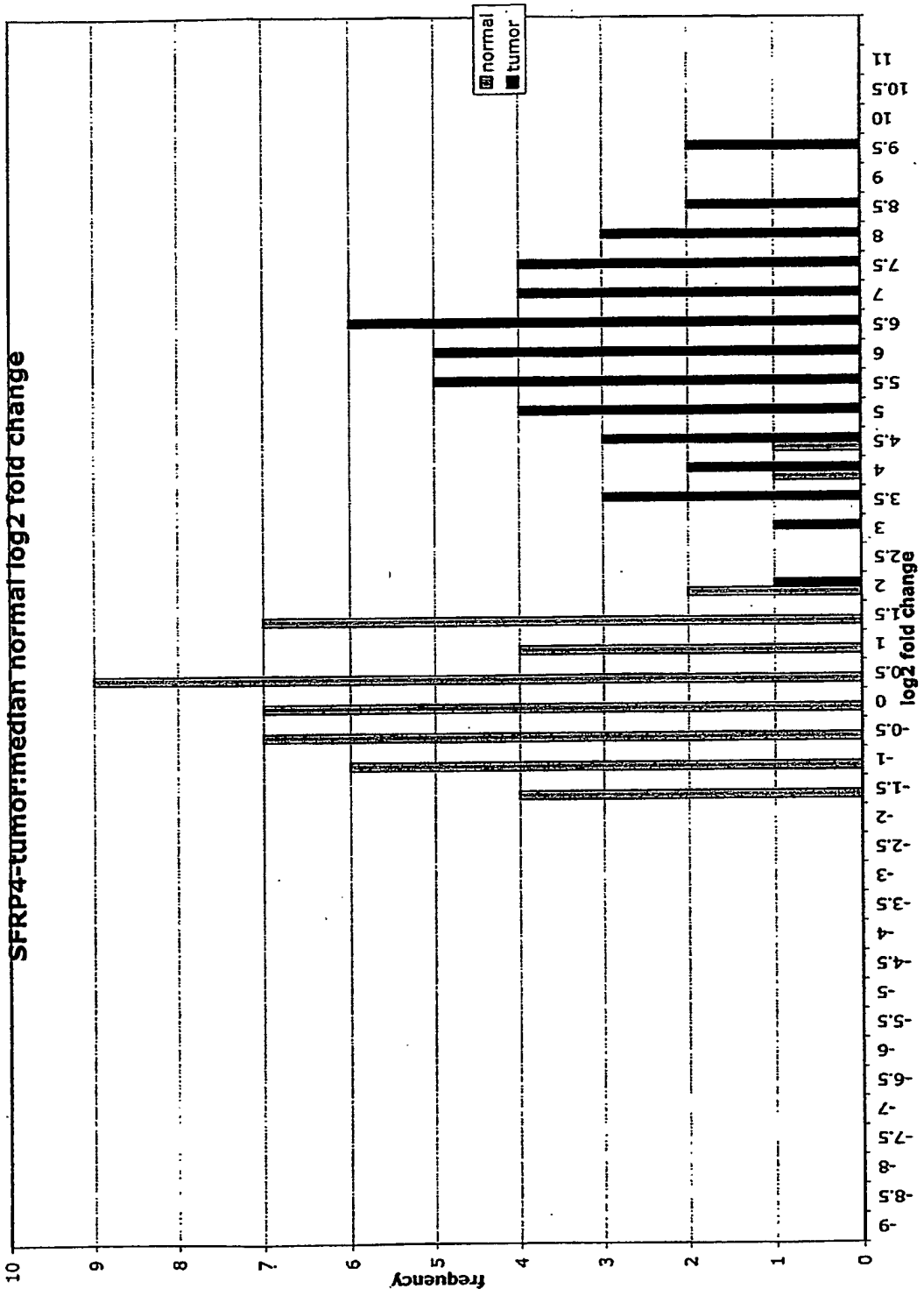

SFRP4-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
10
9
8
7
6
5
4
3
2
1
0
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10
10.5
11

Figure 5(h)

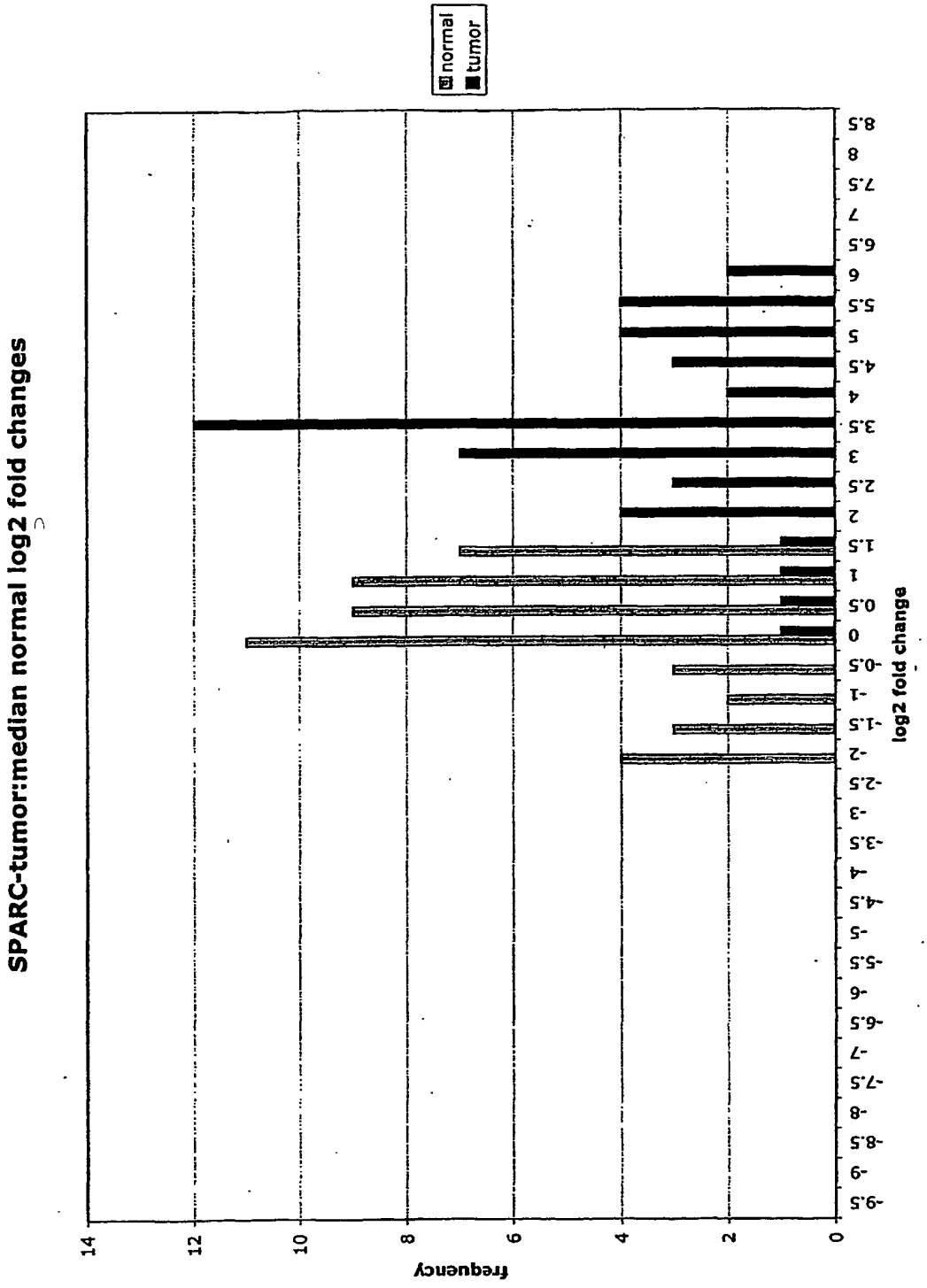

SPARC-tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold change
14
12
10
8
6
4
2
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(i)

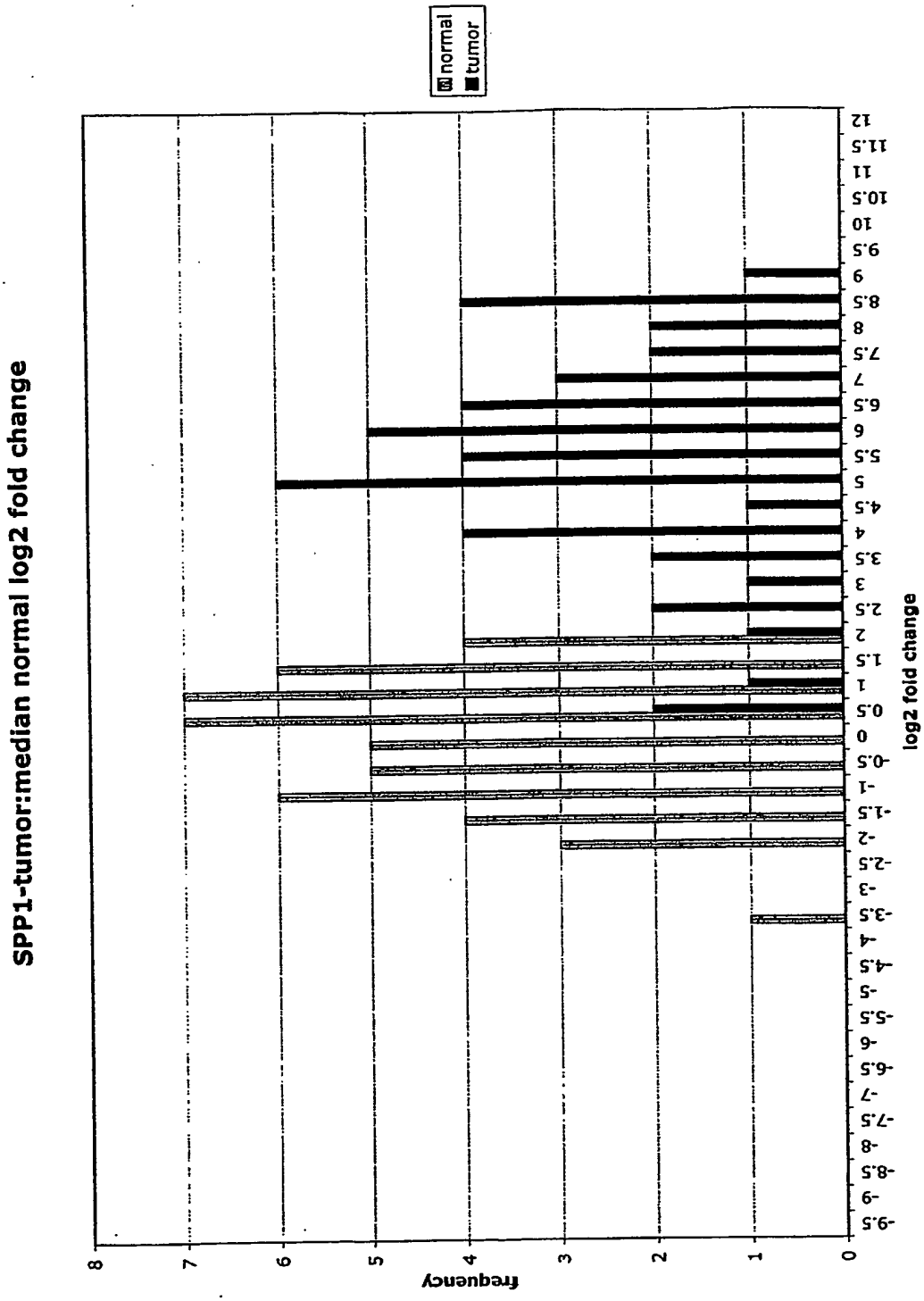

SPP1-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change

Figure 5(j)

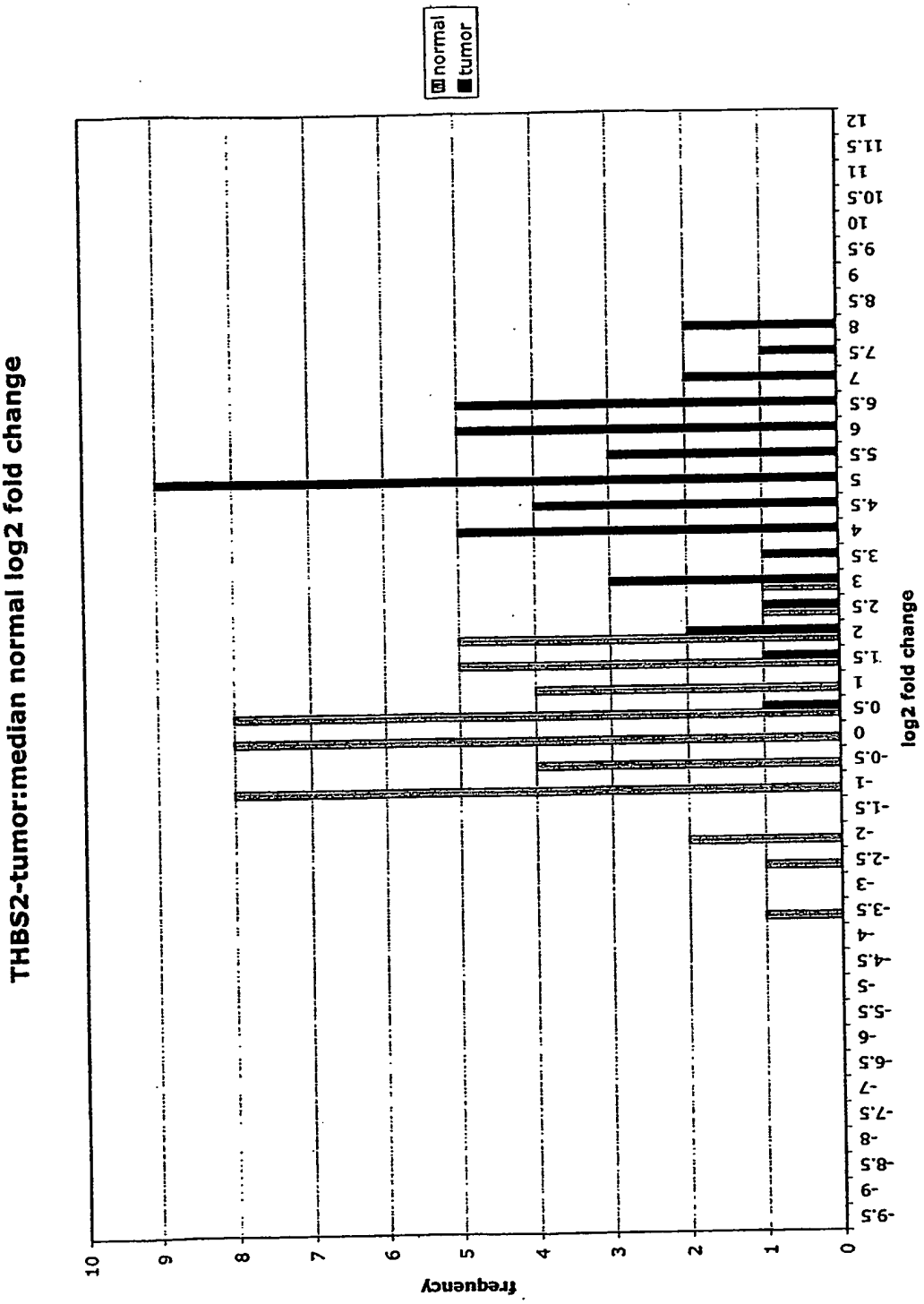

THBS2-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
10
9
8
7
6
5
4
3
2
1
0
12
11.5
11
10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(k)

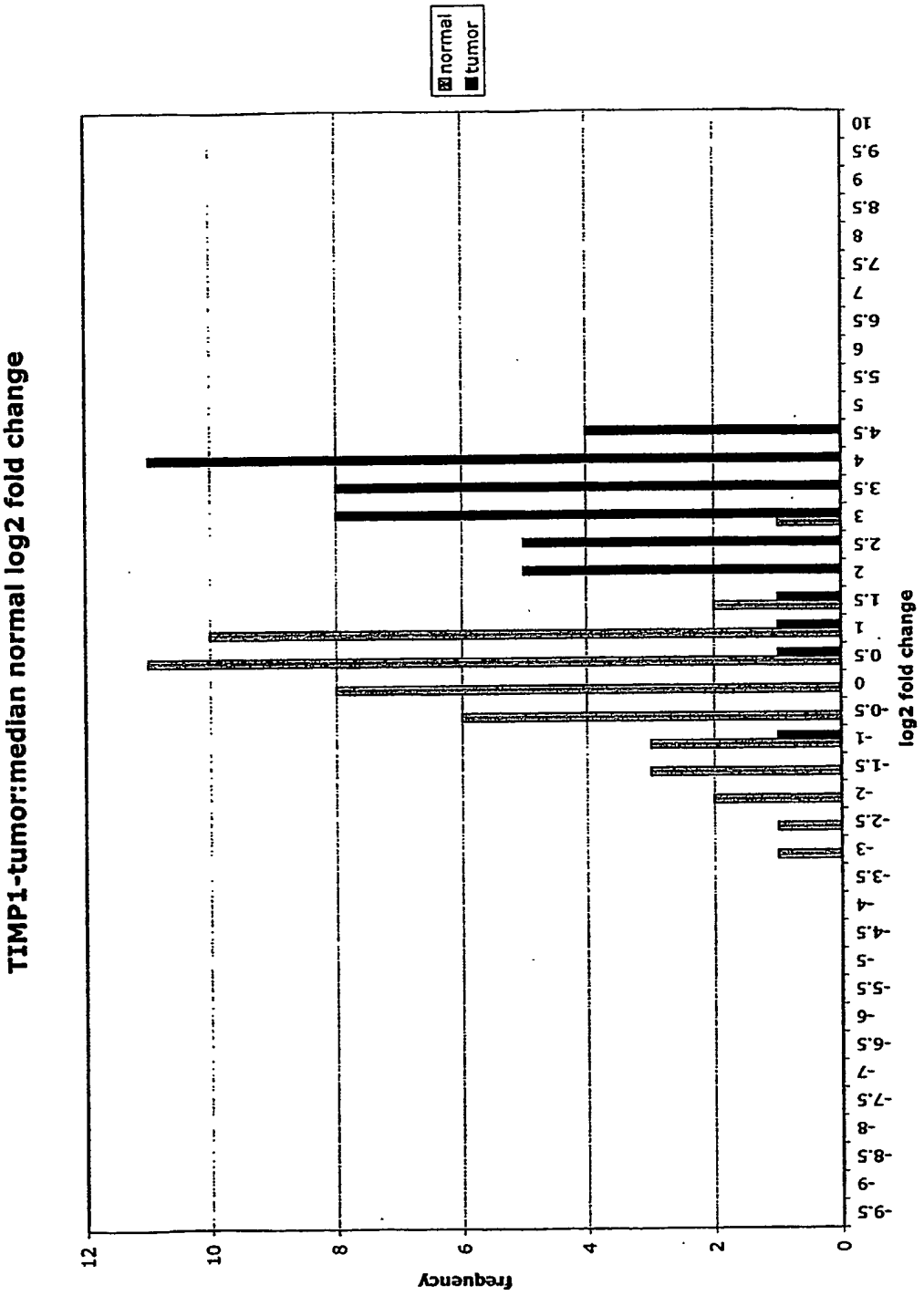

TIMP1-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change

Figure 5(1)

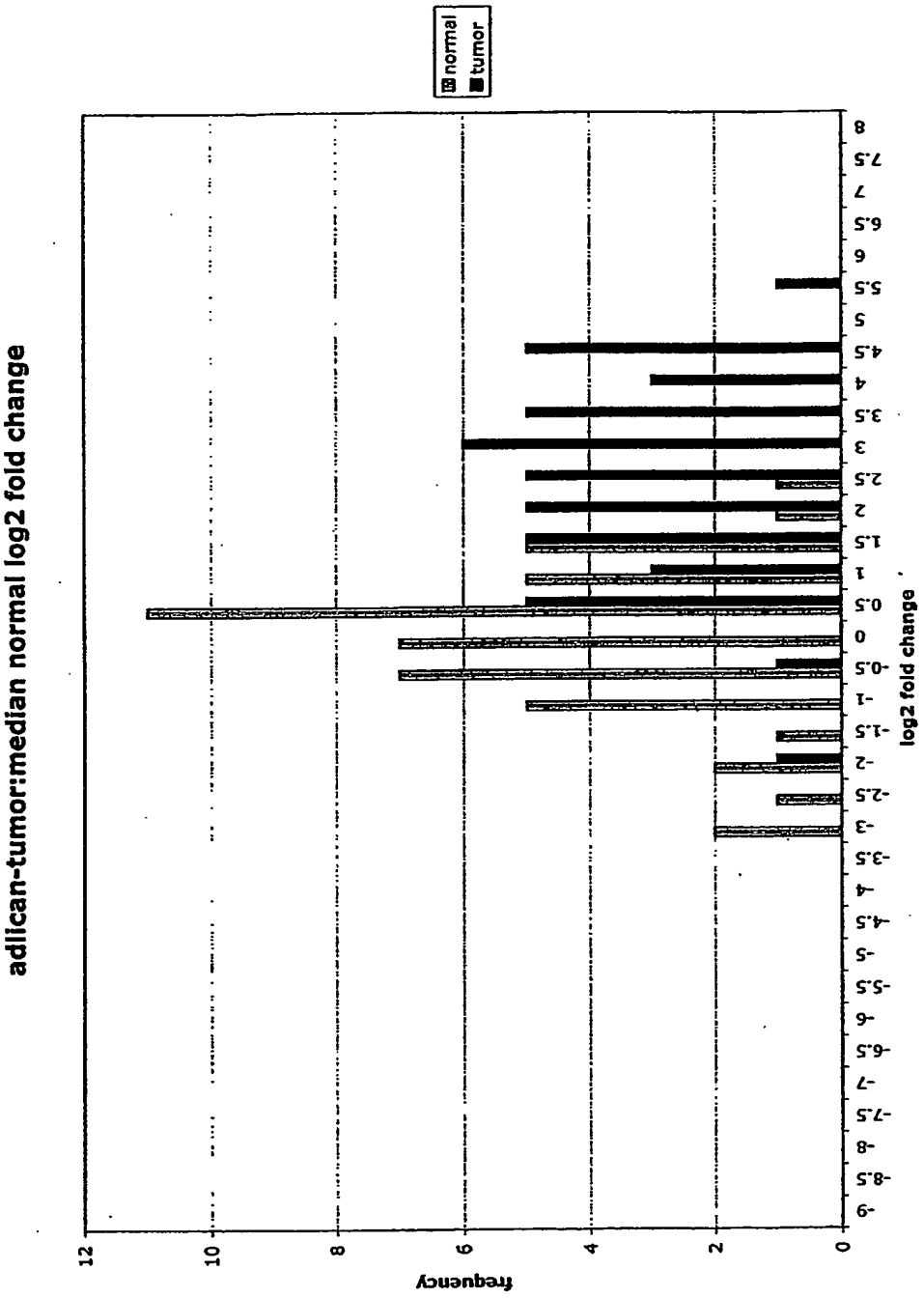

adlican-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change

Figure 5(m)

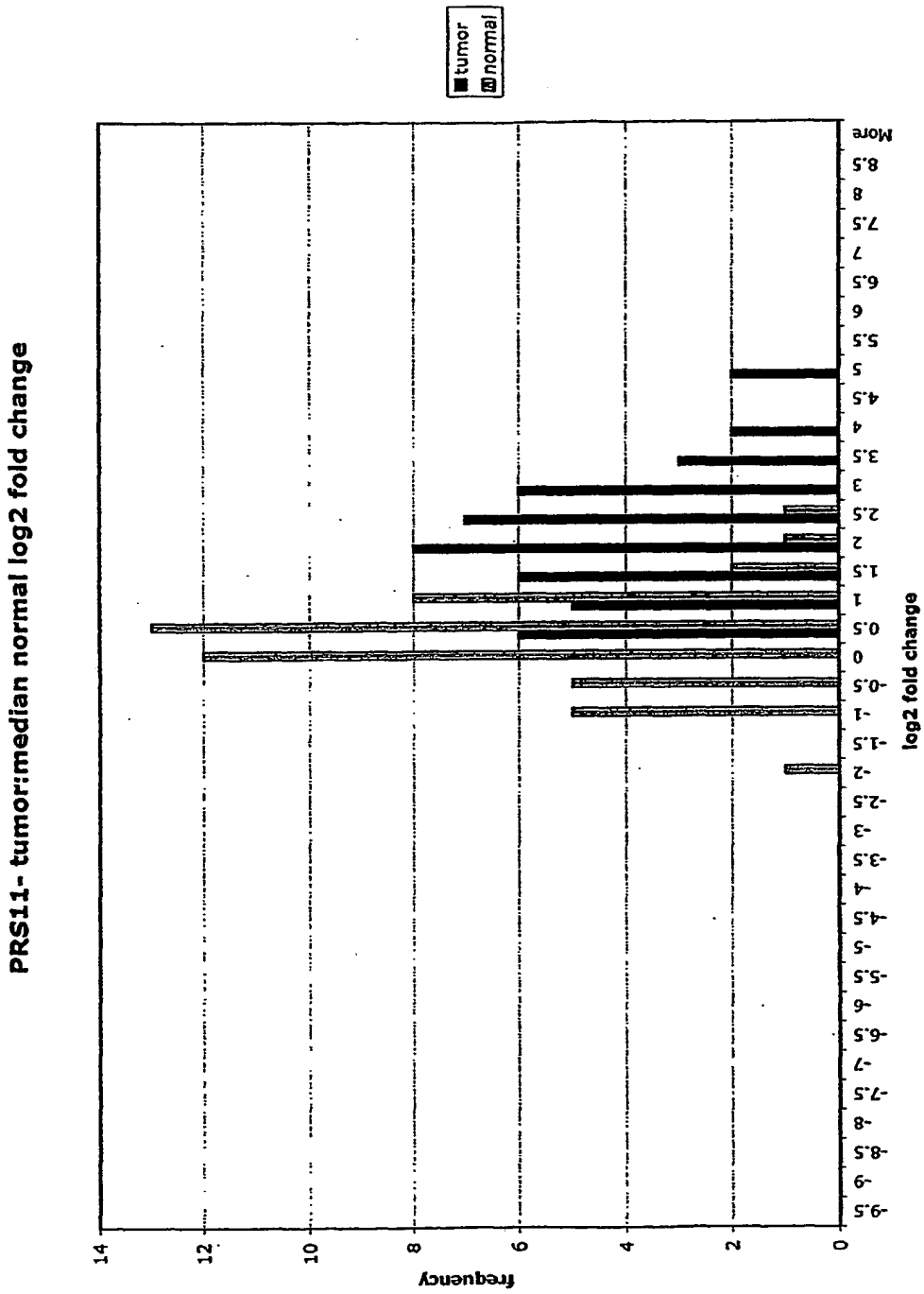

PRS11- tumor:median normal log2 fold change
tumor
normal
Frequency
log2 fold change
14
12
10
8
6
4
2
0
-9.5
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
More

Figure 5(n)

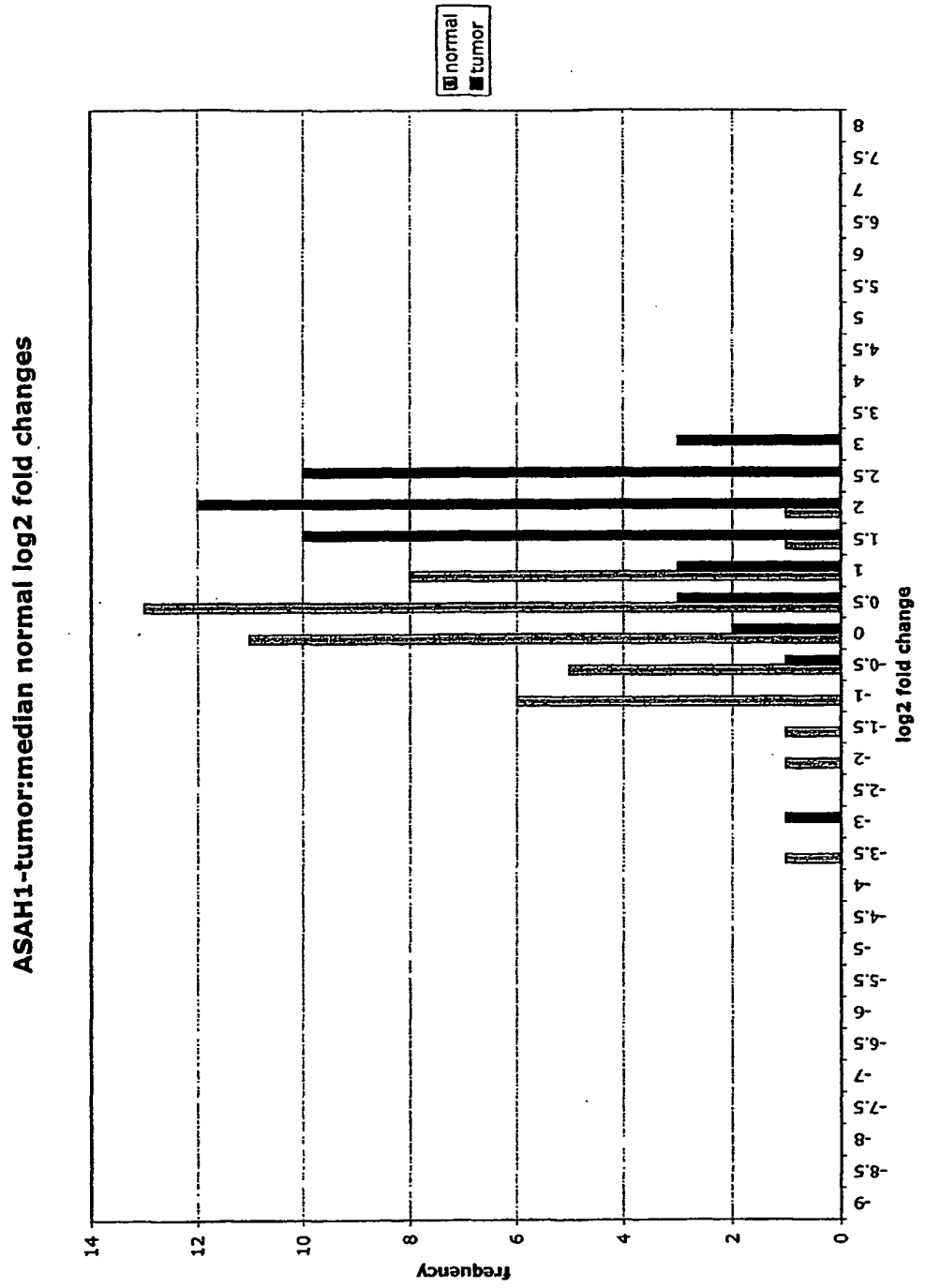

ASAH1-tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold change

Figure 5(o)

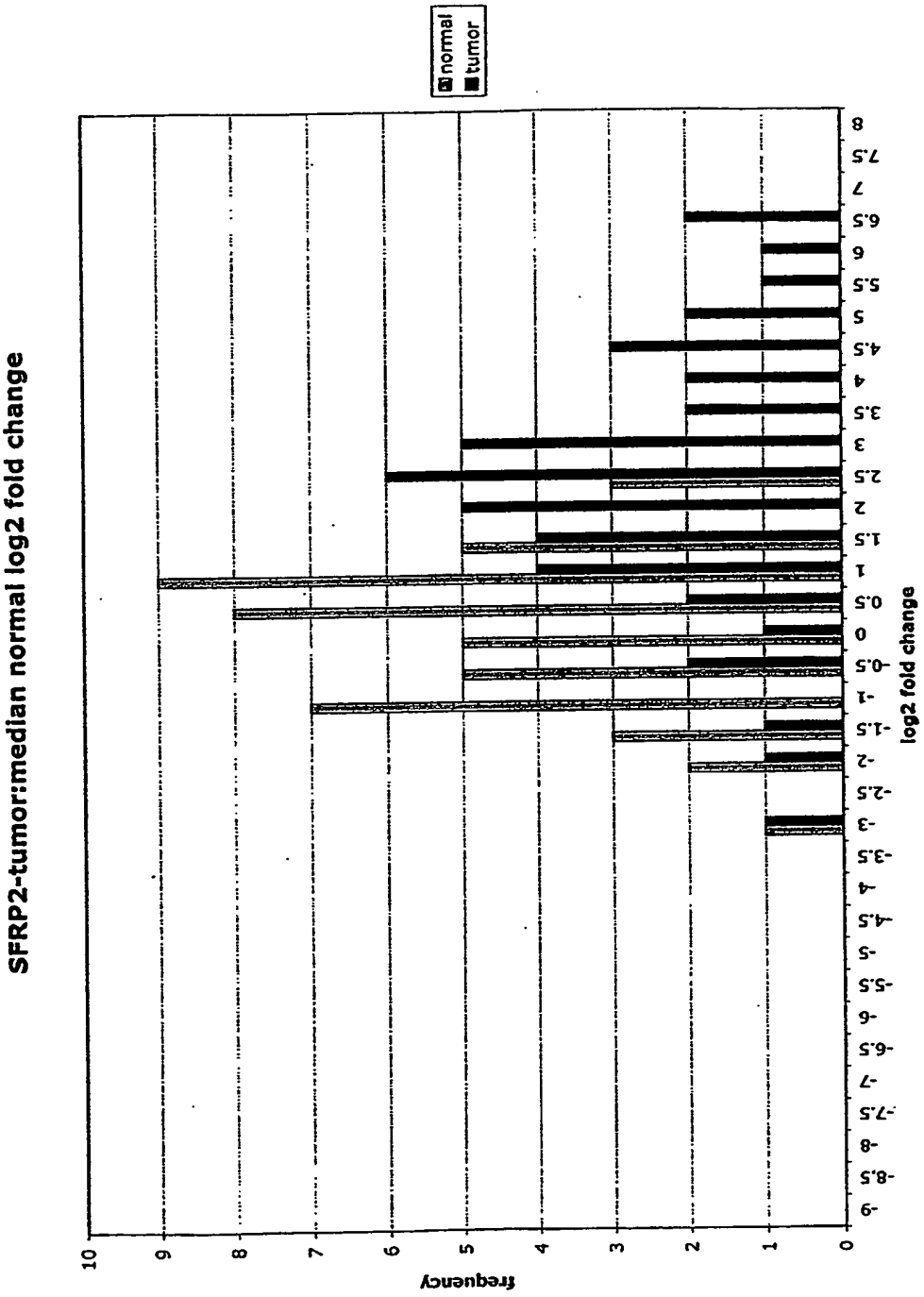

SFRP2-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change

Figure 5(p)

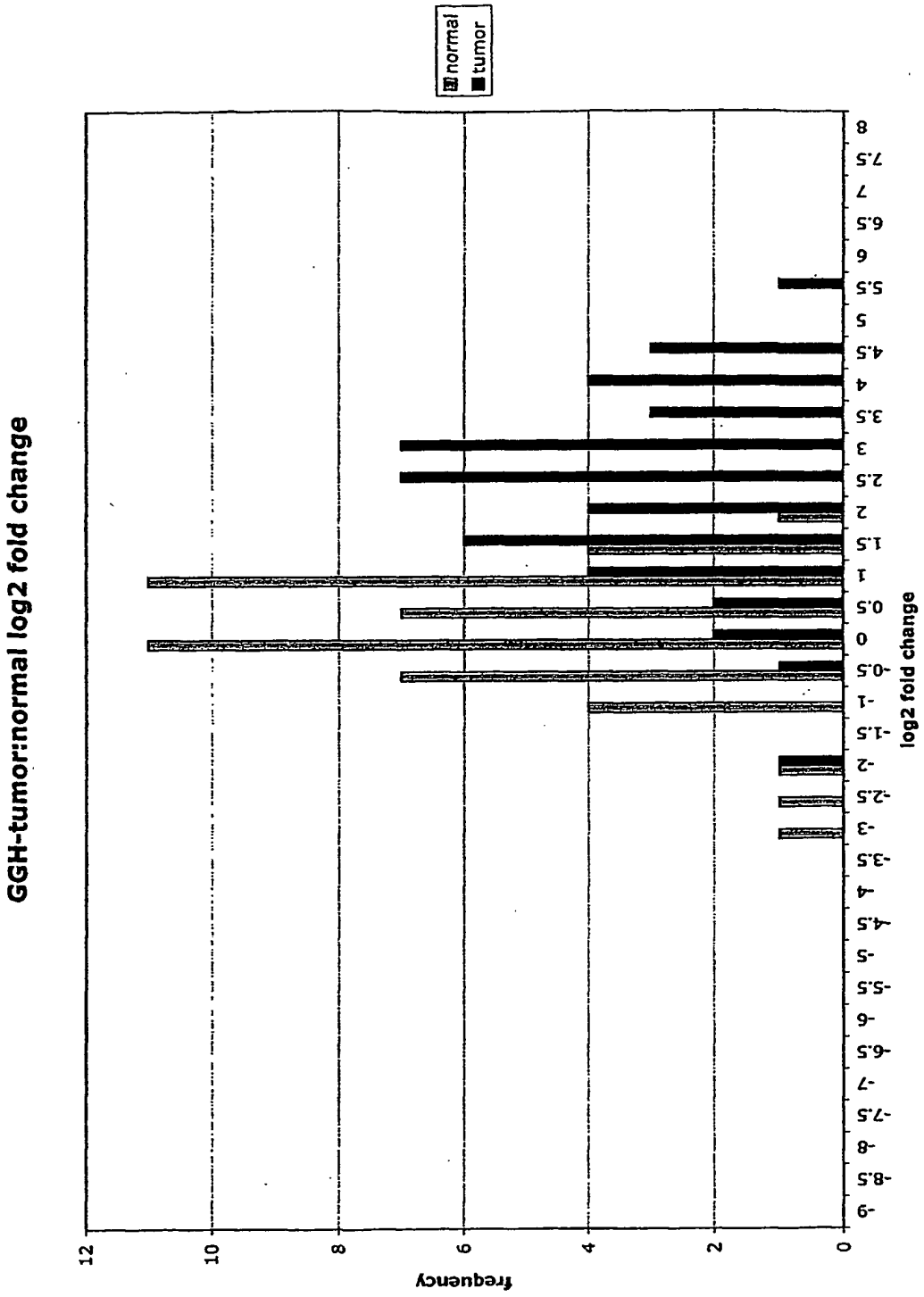

GGH-tumor:normal log2 fold change
normal
tumor
Frequency
log2 fold change

Figure 5(q)

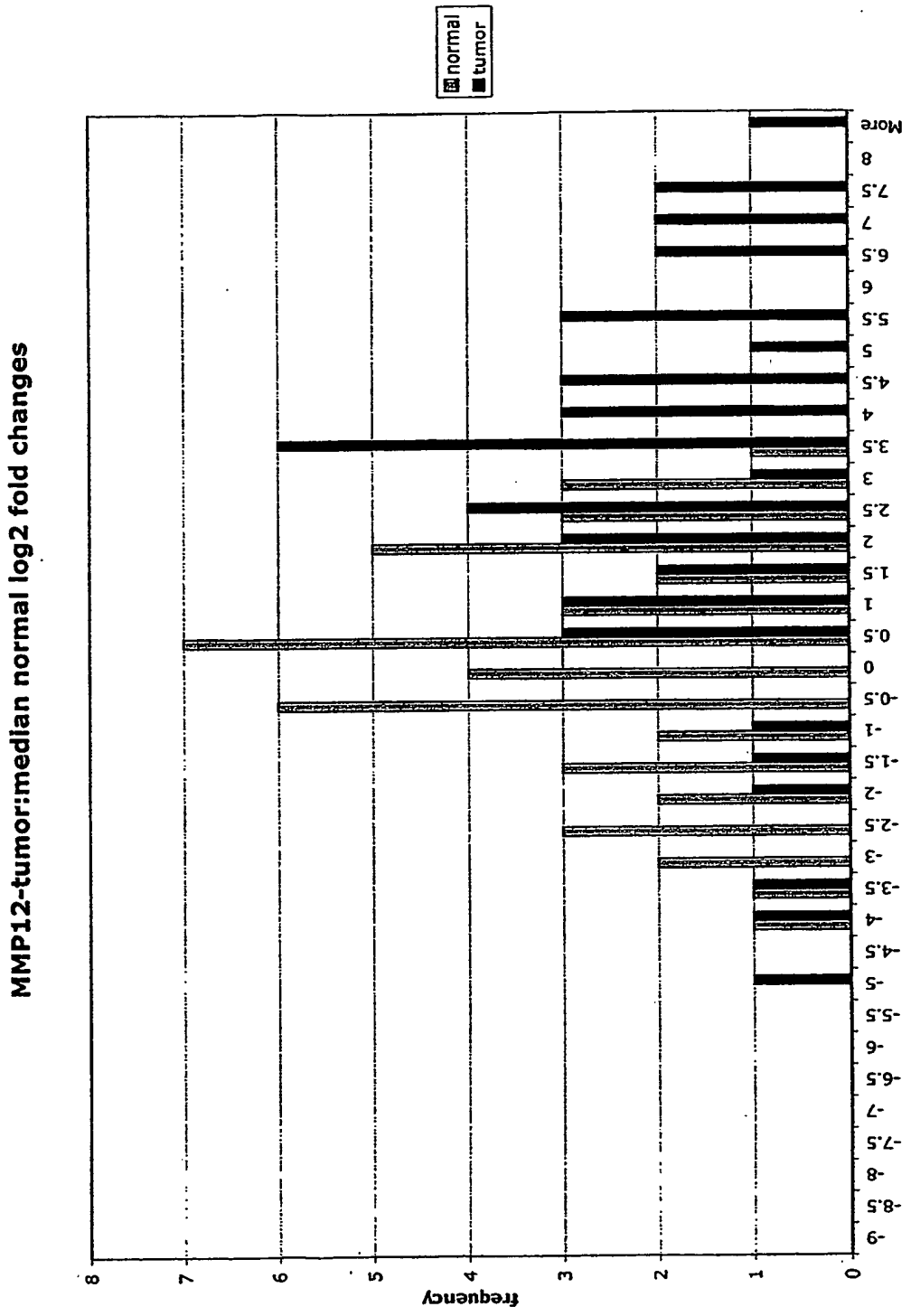

MMP12-tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold changes
0
1
2
3
4
5
6
7
8
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
More

Figure 5(r)

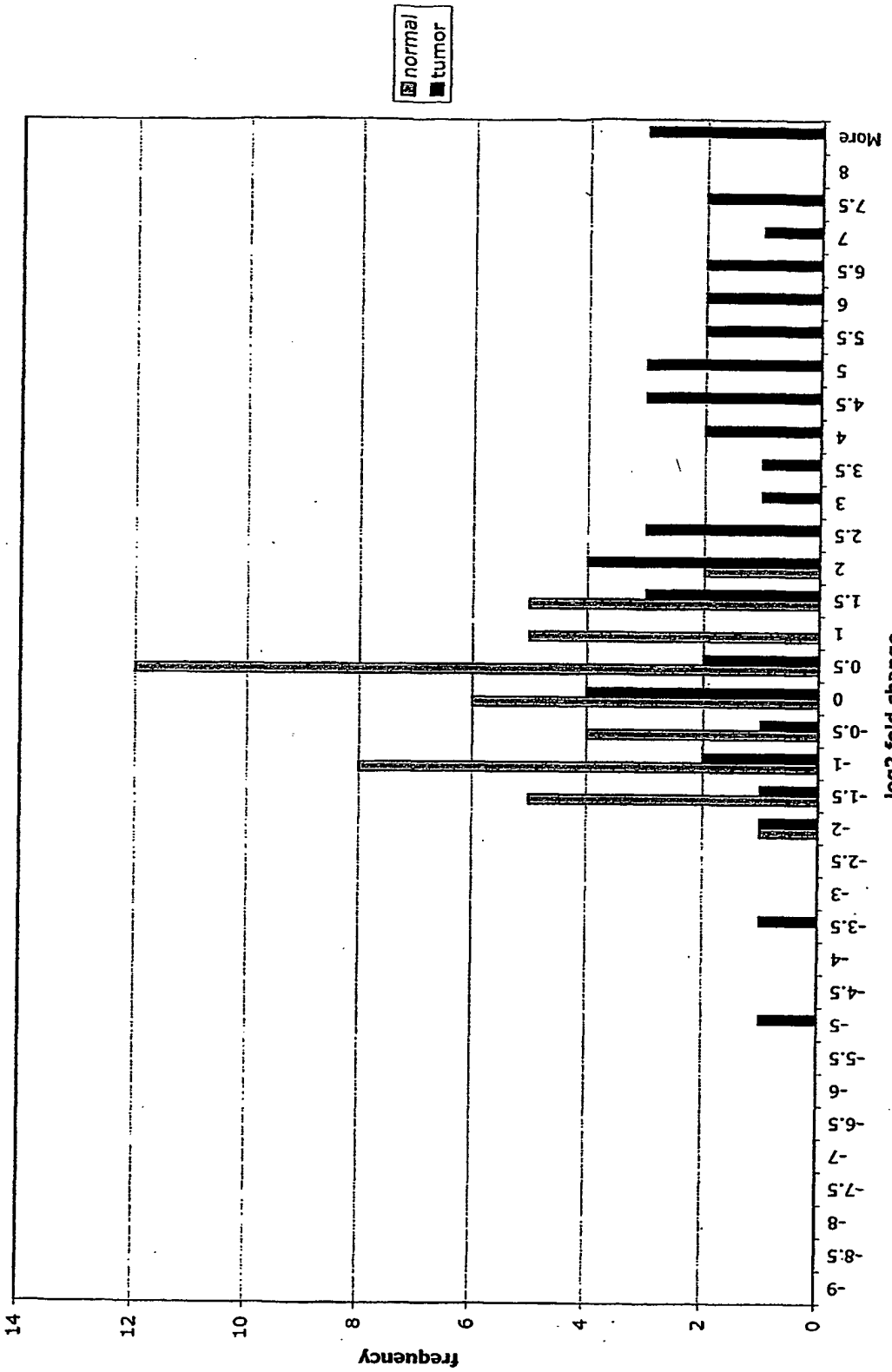

normal
tumor
frequency
log2 fold change
14
12
10
8
6
4
2
0
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
More

Figure 5(s)

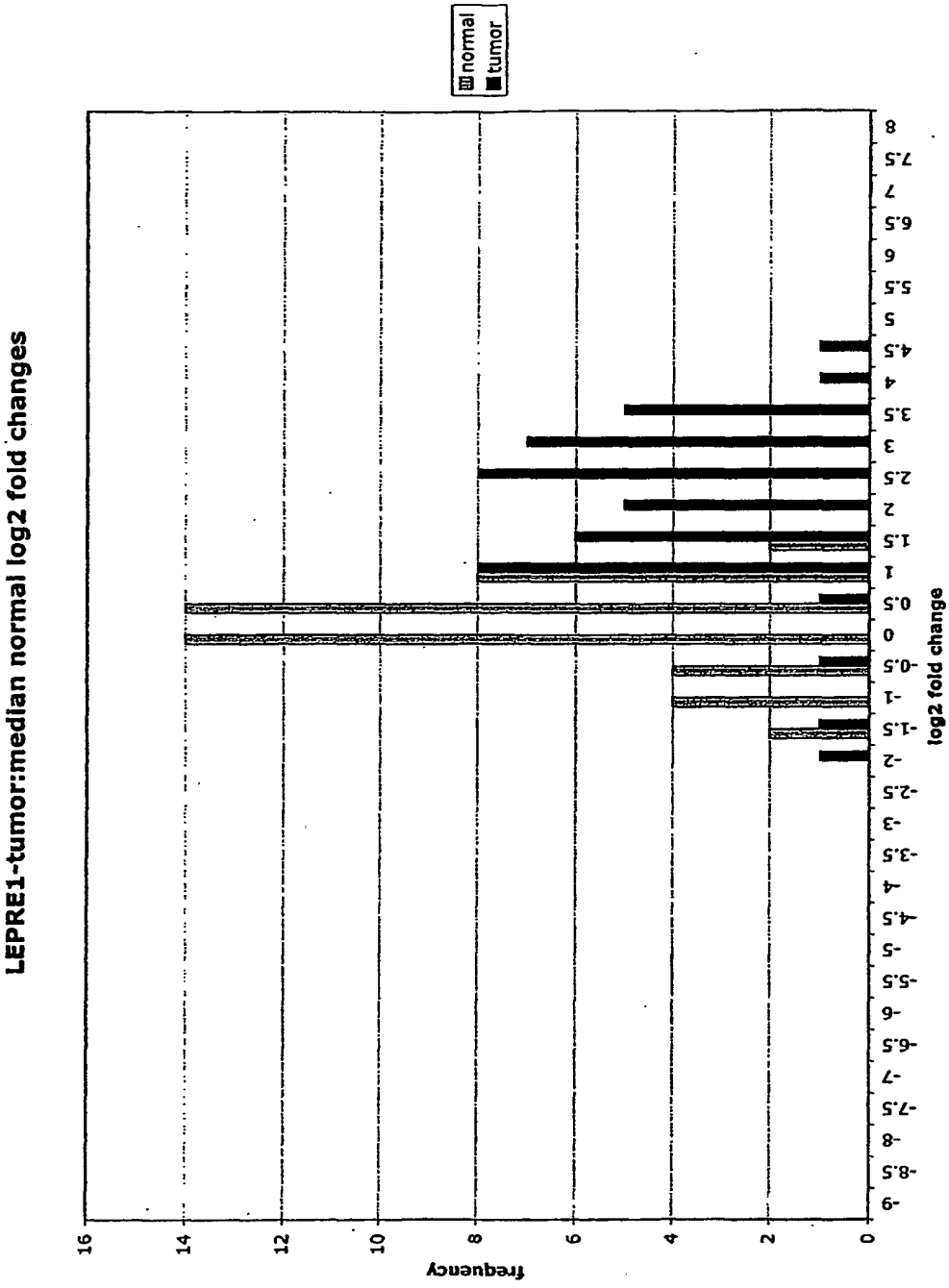

LEPRE1-tumor:median normal log2 fold changes
normal
tumor
frequency
log2 fold change

Figure 5(t)

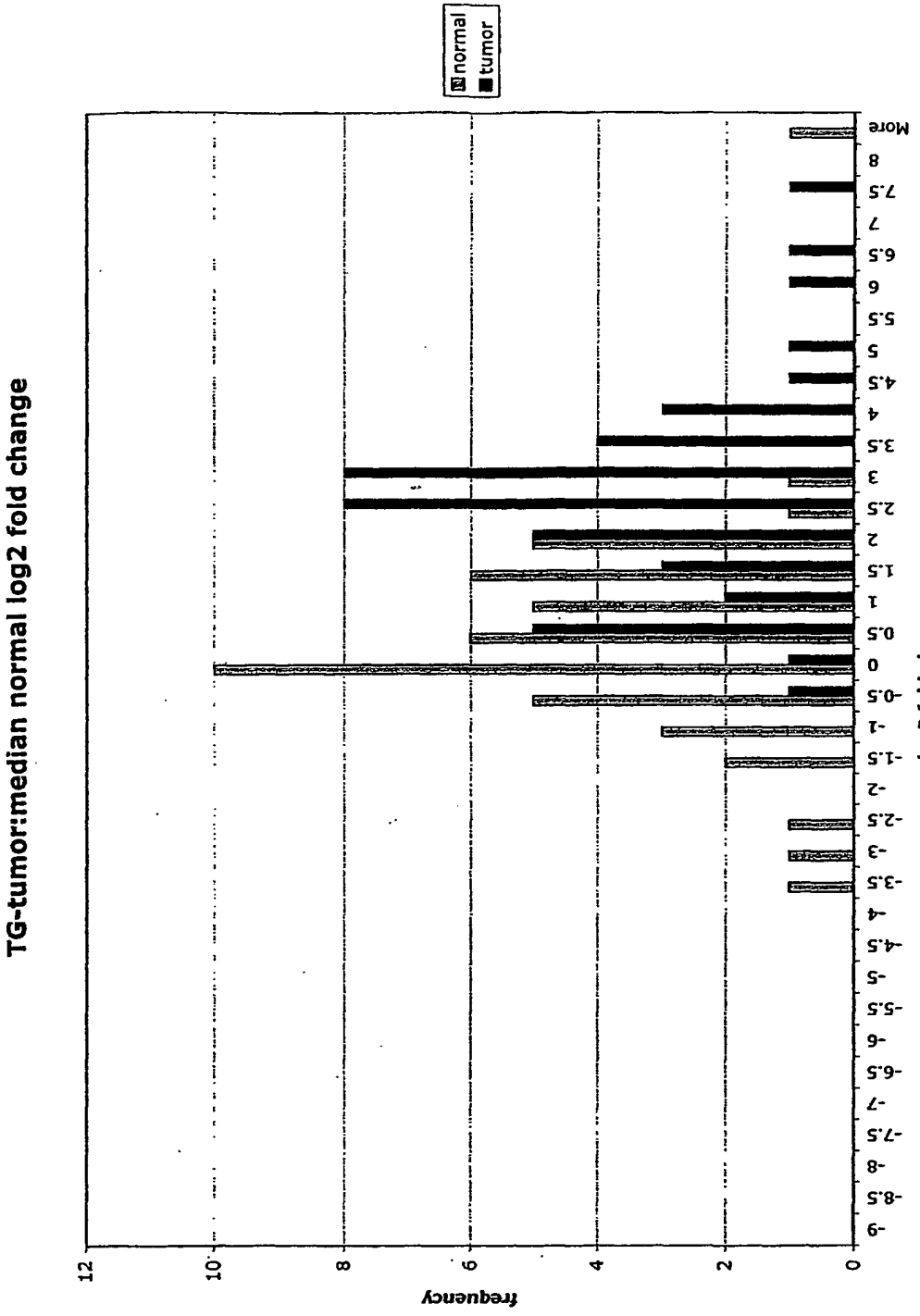

TG-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change
12
10
8
6
4
2
0
-9
-8.5
-8
-7.5
-7
-6.5
-6
-5.5
-5
-4.5
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
More

Figure 5(u)

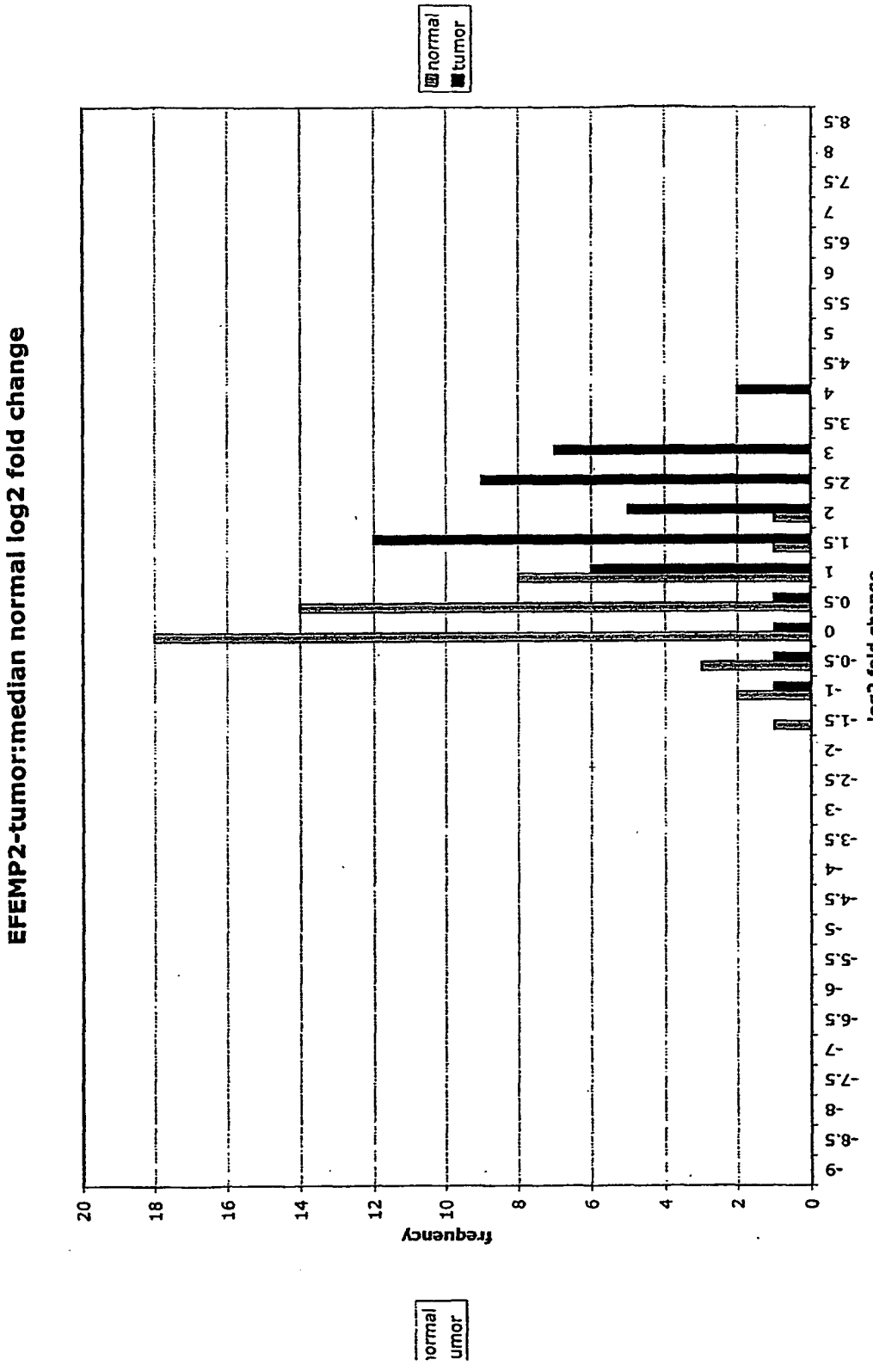

EFEMP2-tumor:median normal log2 fold change
normal
tumor
frequency
log2 fold change

Figure 5(v)

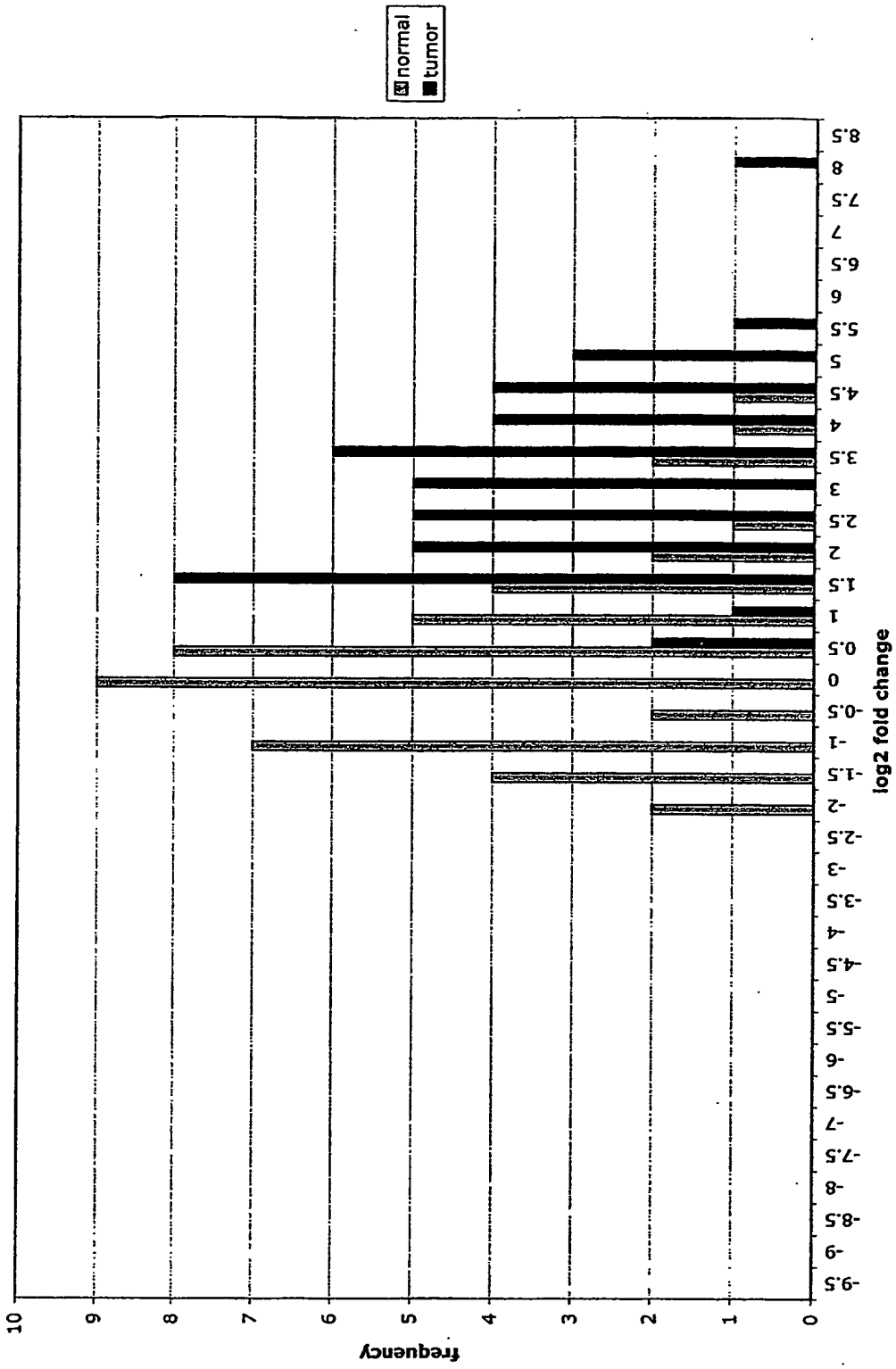

normal
tumor
frequency
log2 fold change
10
9
8
7
6
5
4
3
2
1
0
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
-7.5
-8
-8.5
-9
-9.5

Figure 5(w)

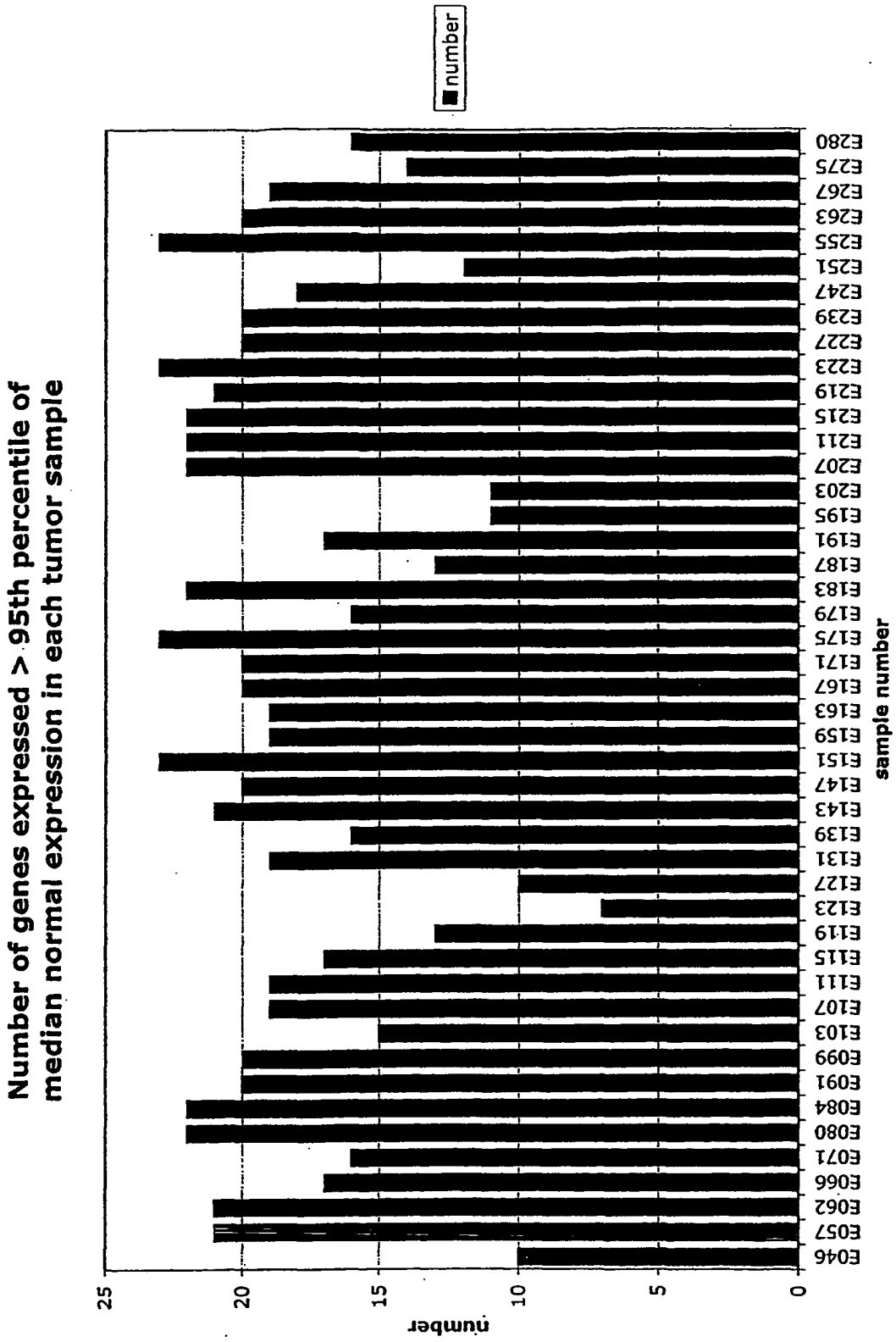

Number of genes expressed > 95th percentile of median normal expression in each tumor sample
number
sample number
number
0
5
10
15
20
25
E046
E057
E062
E066
E071
E080
E084
E091
E099
E103
E107
E111
E115
E119
E123
E127
E131
E139
E143
E147
E151
E159
E163
E167
E171
E175
E179
E183
E187
E191
E195
E203
E207
E211
E215
E219
E223
E227
E239
E247
E251
E255
E263
E267
E275
E280

Figure 6

Fig.7a Relative expression of markers in tumor and normal samples compared to CEA

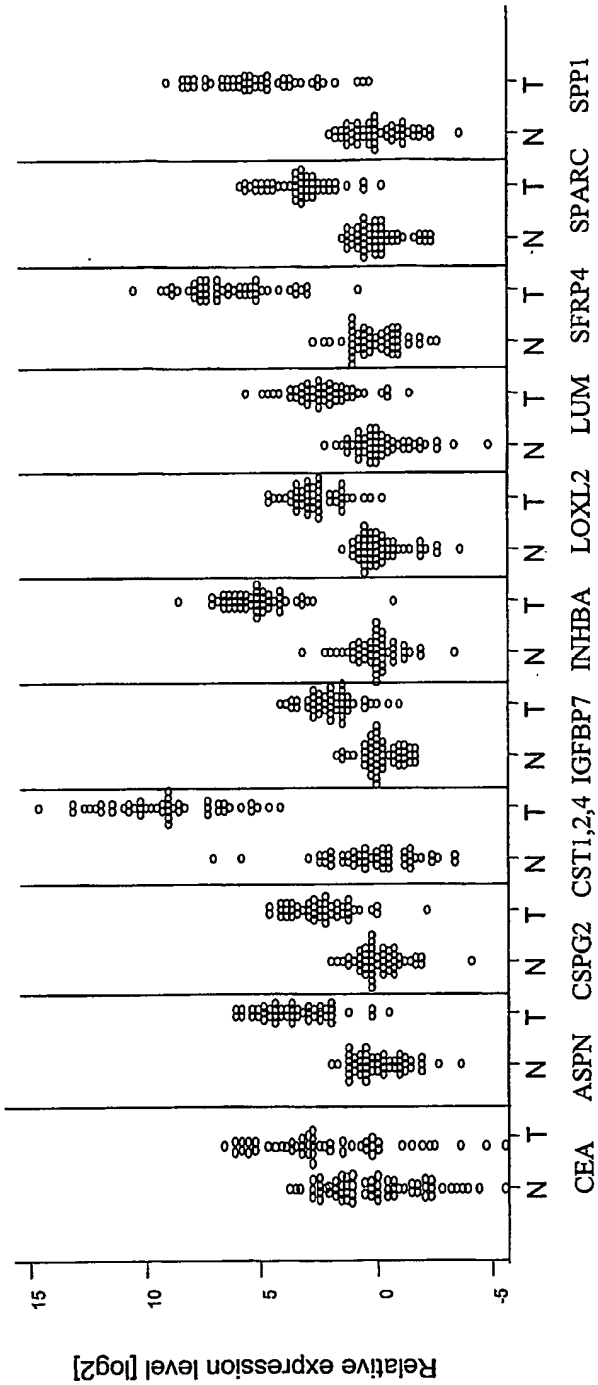


Fig. 7b

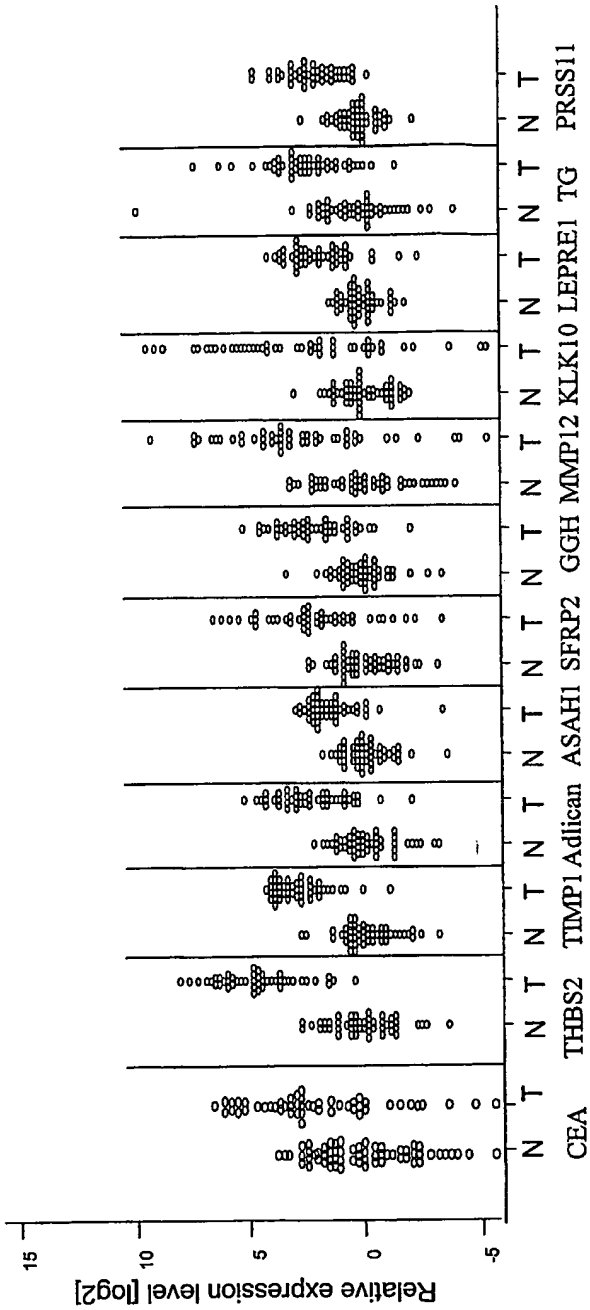

Relative expression level [log2]
15
10
5
0
-5
N T N T N T N T N T N T N T N T N T N T N T N T N T
CEA THBS2 TIMP1 Adlican ASAH1 SFRP2 GGH MMP12 KLK10 LEPRE1 TG PRSS11

Fig. 7c

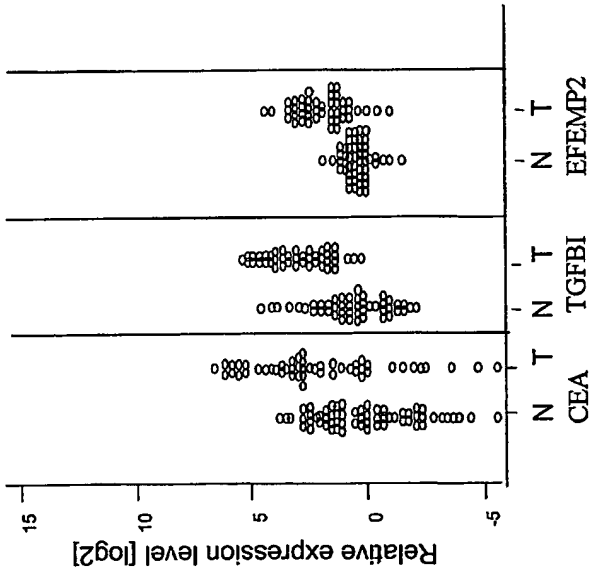
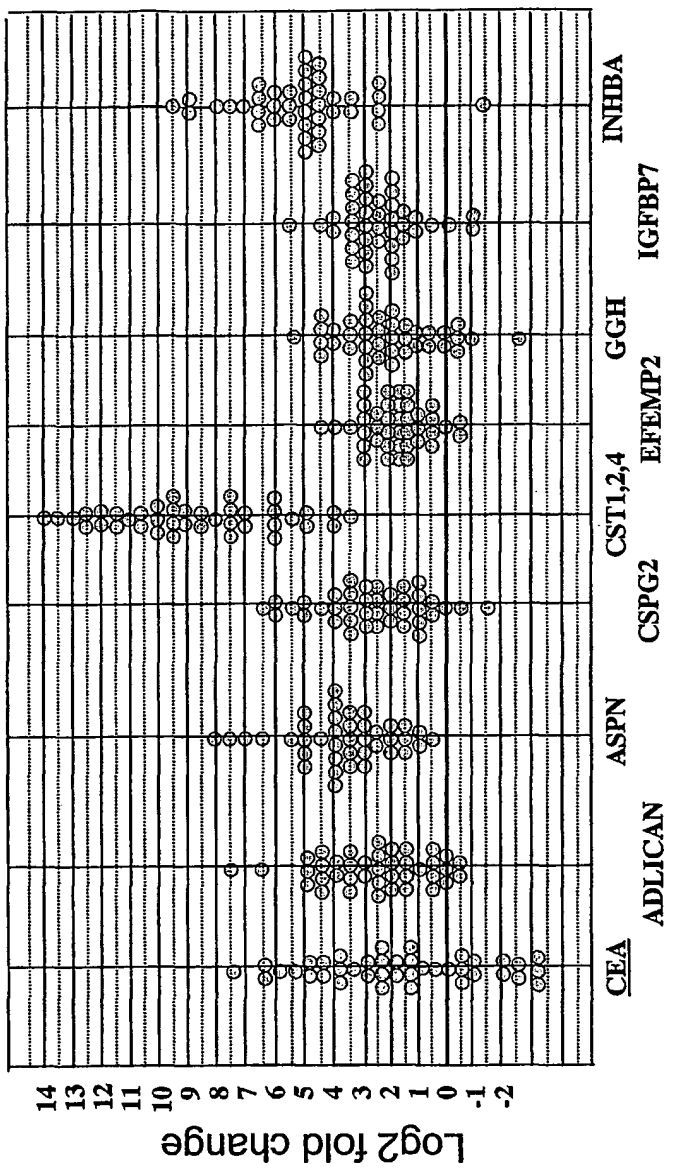

Relative expression level [log2]
15
10
5
0
-5
N T N T N T
CEA TGFBI EFEMP2

Fig. 8. Quantitative RT-PCR: expression in paired tumor and non-malignant samples of selected gastric cancer markers

name	symbol	median T:N fold change	maximum T:N fold change	% tumor samples with expression >paired non-malignant sample	
adlican		5	146	88	
asporin (lrr class 1)	ASPN	11	198	100	
chondroitin sulfate proteoglycan 2 (versican)	CSPG2	5	68	93	
cystatins SN, SA & S	CST1, 2, 4	498	11911	100	
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	3	17	93	
gamma-glutamyl hydrolase	GGH	4	34	83	
inhibin beta A chain	INHBA	27	630	95	
insulin-like growth factor binding protein 7	IGFBP7	5	38	93	
kallikrein 10	KLK10	7	519	78	
leucine proline-enriched proteoglycan 1(leprecan 1)	LEPRE1	4	23	85	
lumican	LUM	5	68	90	
lysyl oxidase-like 2	LOXL2	7	53	95	
matrix metalloproteinase 12	MMP12	9	468	85	
metalloproteinase inhibitor 1	TIMP1	6	103	95	
n-acylsphingosine amidohydrolase	ASAH1	3	15	88	
osteopontin	SPP1	36	626	98	
secreted frizzled-related protein 2	SFRP2	5	48	83	
secreted frizzled-related protein 4	SFRP4	54	375	100	
secreted protein, acidic, cysteine rich	SPARC	10	66	95	
serine protease 11 (IGF binding)	PRSS11	4	63	90	
thrombospondin 2	THBS2	23	452	98	
thyroglobulin	TG	4	174	93	
transforming growth factor B-induced	TGFBI	5	78	95	
cell growth regulatory factor with EF-hand domain	CGR11	3	33	75	
serine (or cysteine) proteinase inhibitor H1	SERPINH1	10	51	98	
matrix metalloproteinase 12	MMP2	2	46	83	
proprotein convertase subtilisin/kexin type 5	PCSK5	2	63	80	
serine (or cysteine) proteinase inhibitor B5	SERPINB5	5	861	73	
transforming growth factor β1	TGFB1	3	16	88	
carcinoembryonic antigen (CEA)	CEACAM5	3	177	68	

Fig. 9a Relative tumor:normal fold changes in paired tumor/normal gastric samples



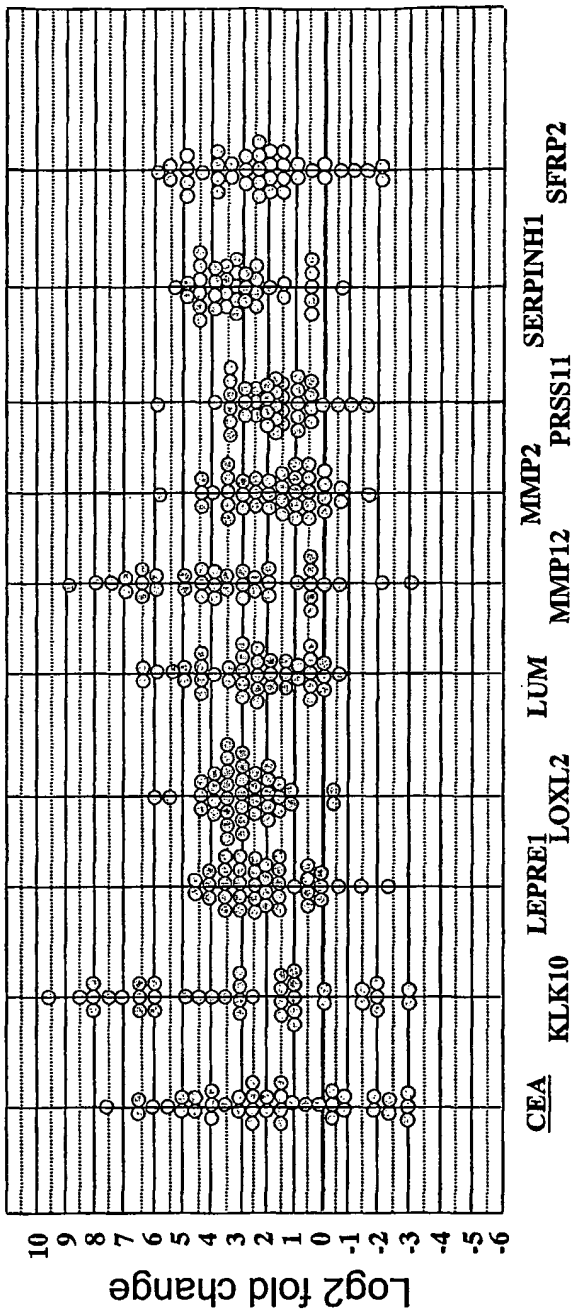
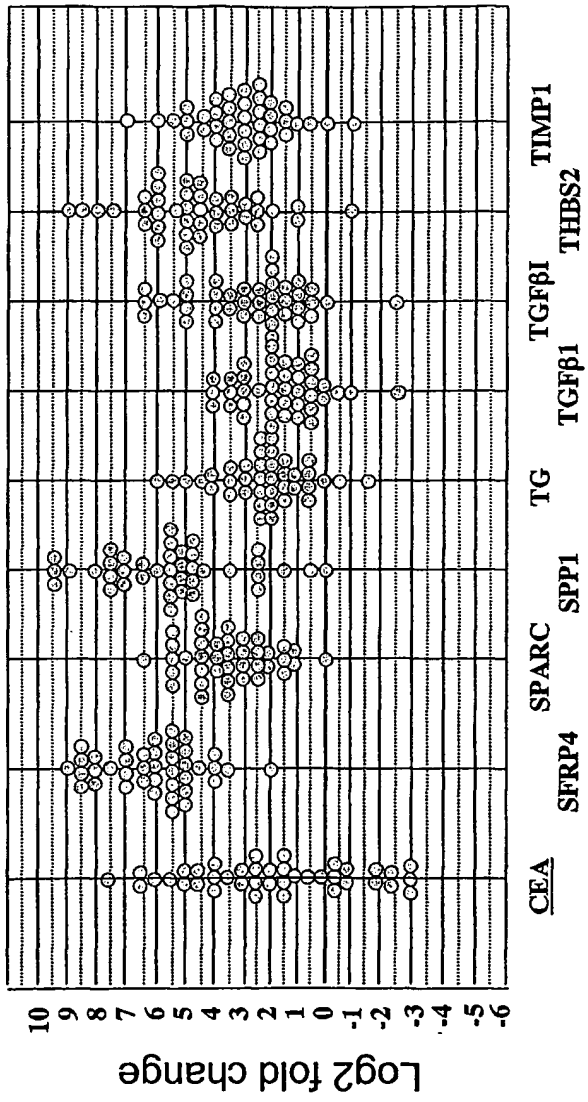

Log2 fold change
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
CEA
KLK10
LEPRE1
LOXL2
LUM
MMP12
MMP2
PRSS11
SERPINH1
SFRP2

Fig. 9b

Fig. 9c


Log2 fold change
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
CEA
SFRP4
SPARC
SPP1
TG
TGFβ1
TGFβI
THBS2
TIMP1

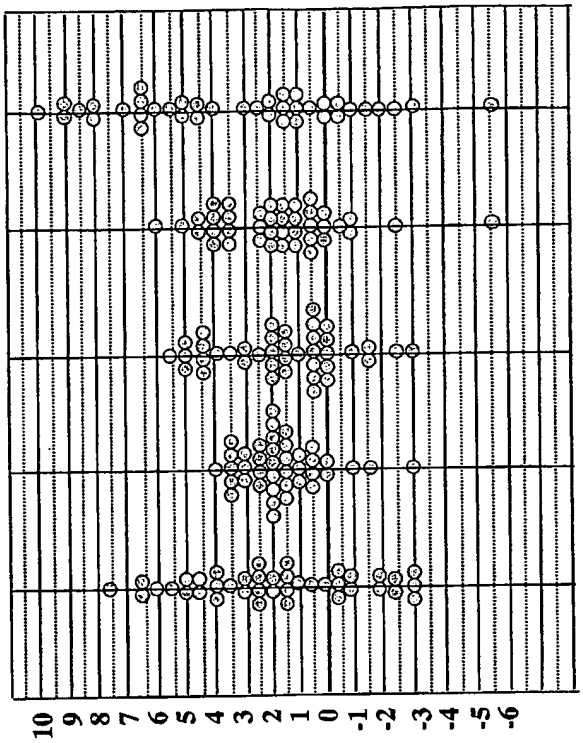
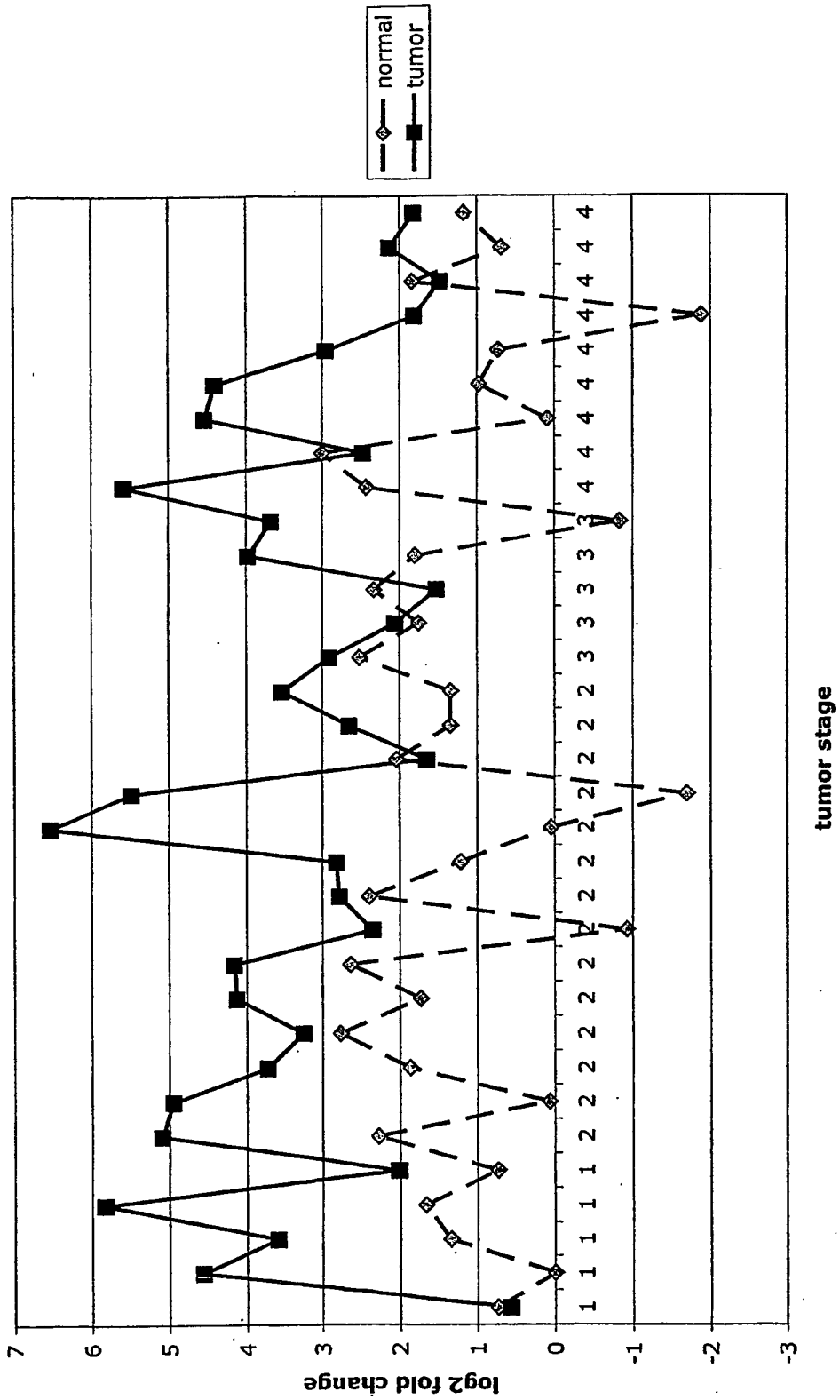

Log2 fold change
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
CEA
ASAH1
CGR11
PCSK5
SERPINB5

Fig. 9d

Fig. 10a adlican



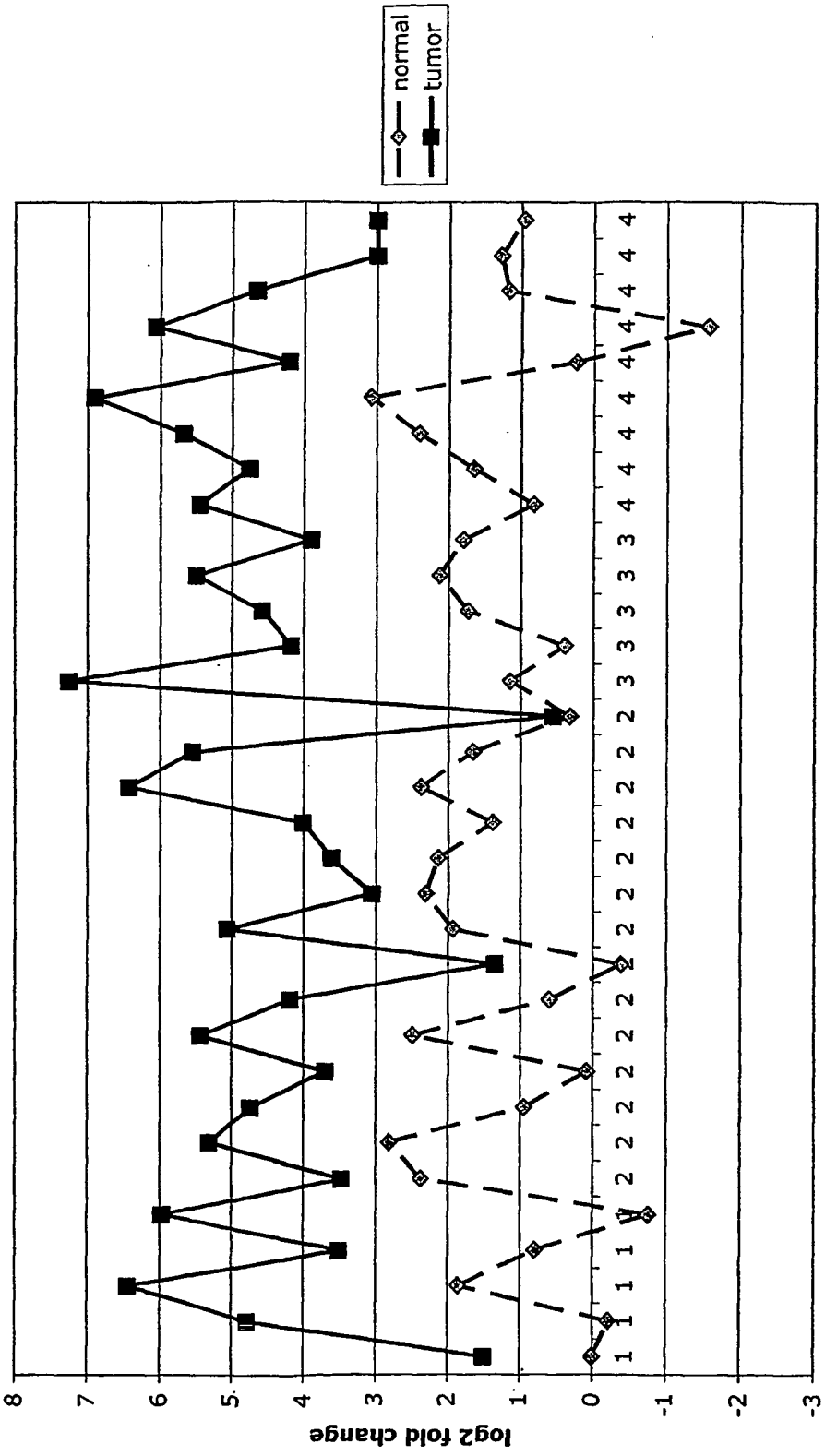
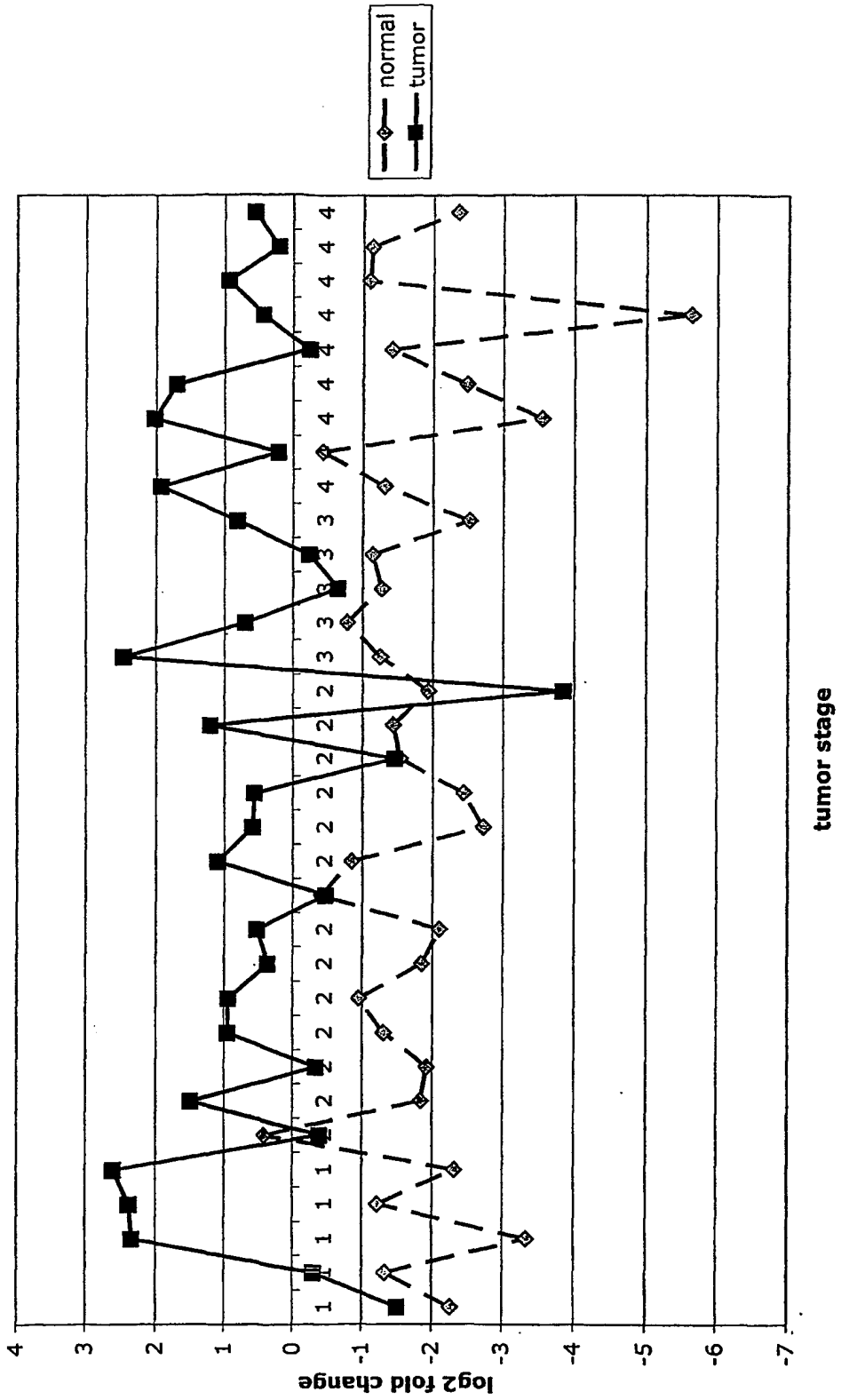


Fig. 10b ASPN

Fig. 10c CSPG2



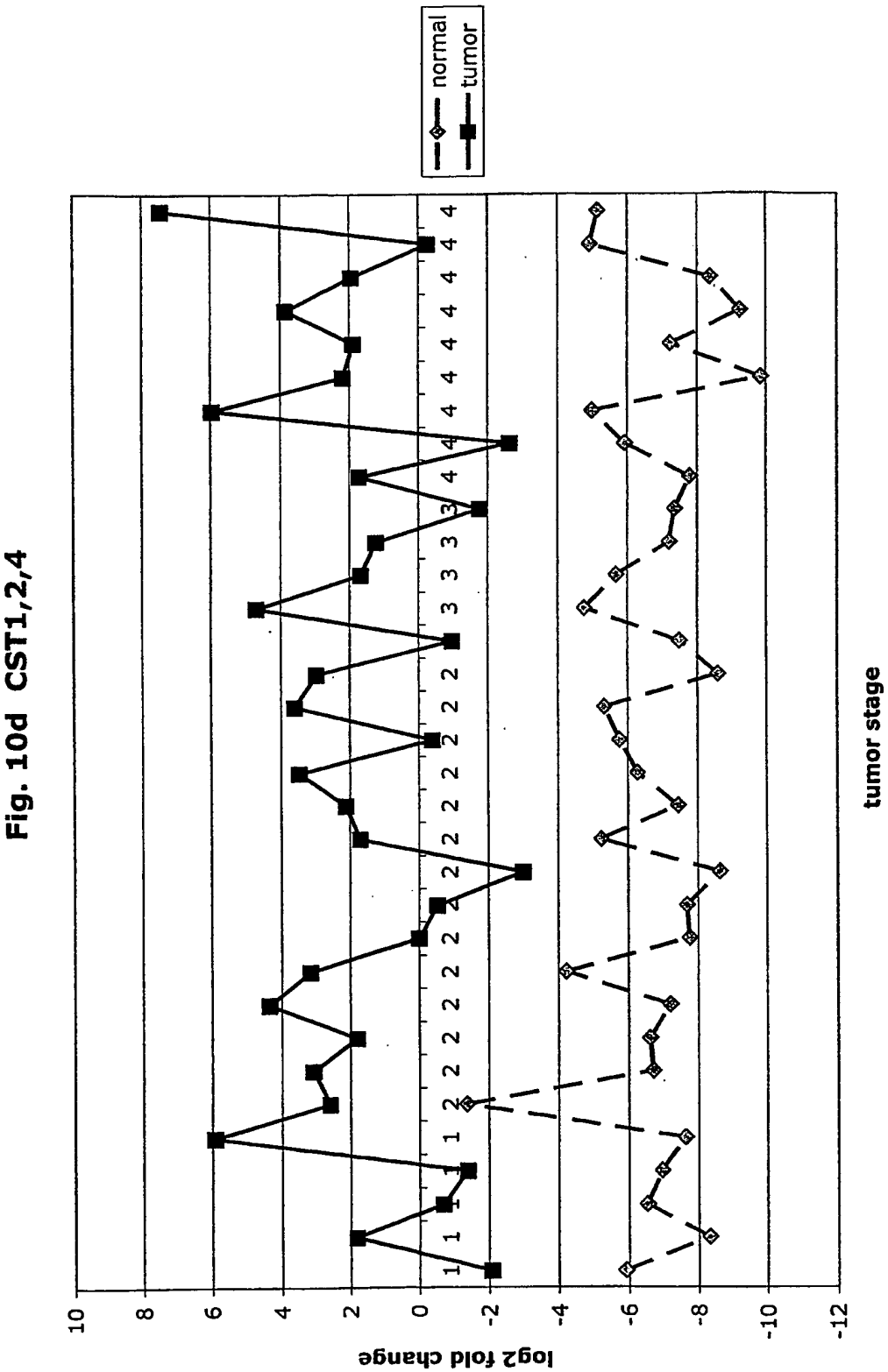


Fig. 10d CST1,2,4

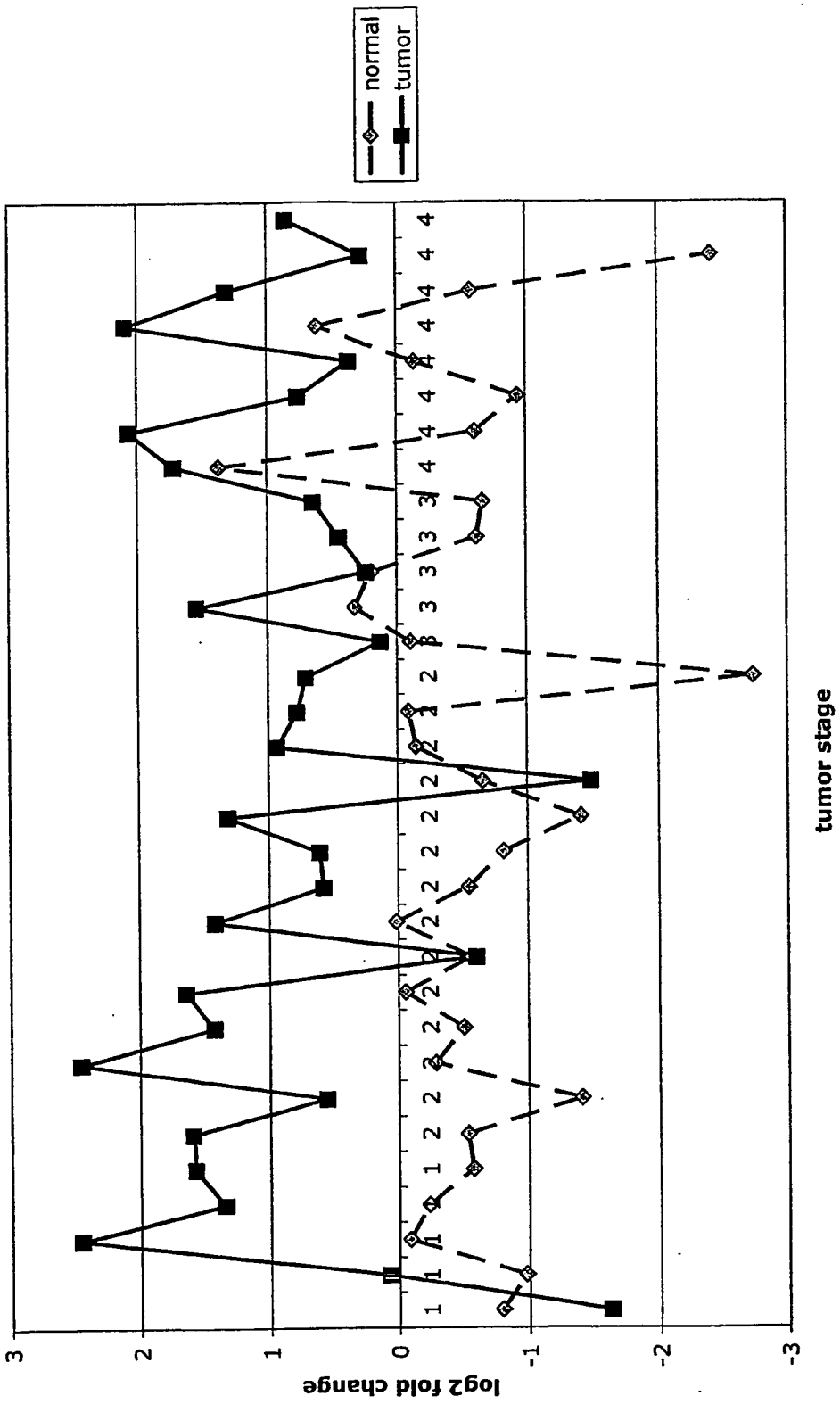


Fig. 10e EFEMP2

Fig. 10f GGH

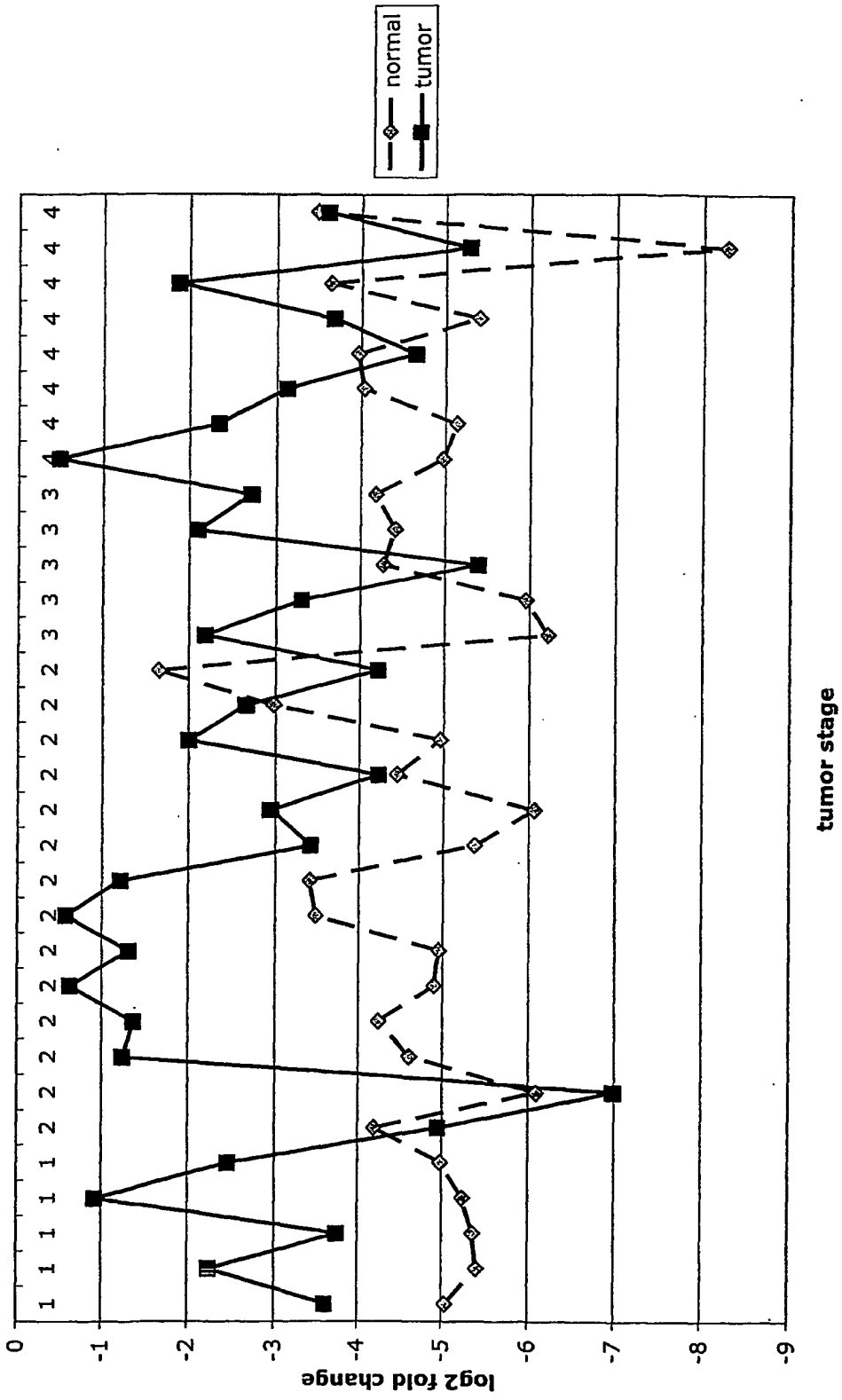
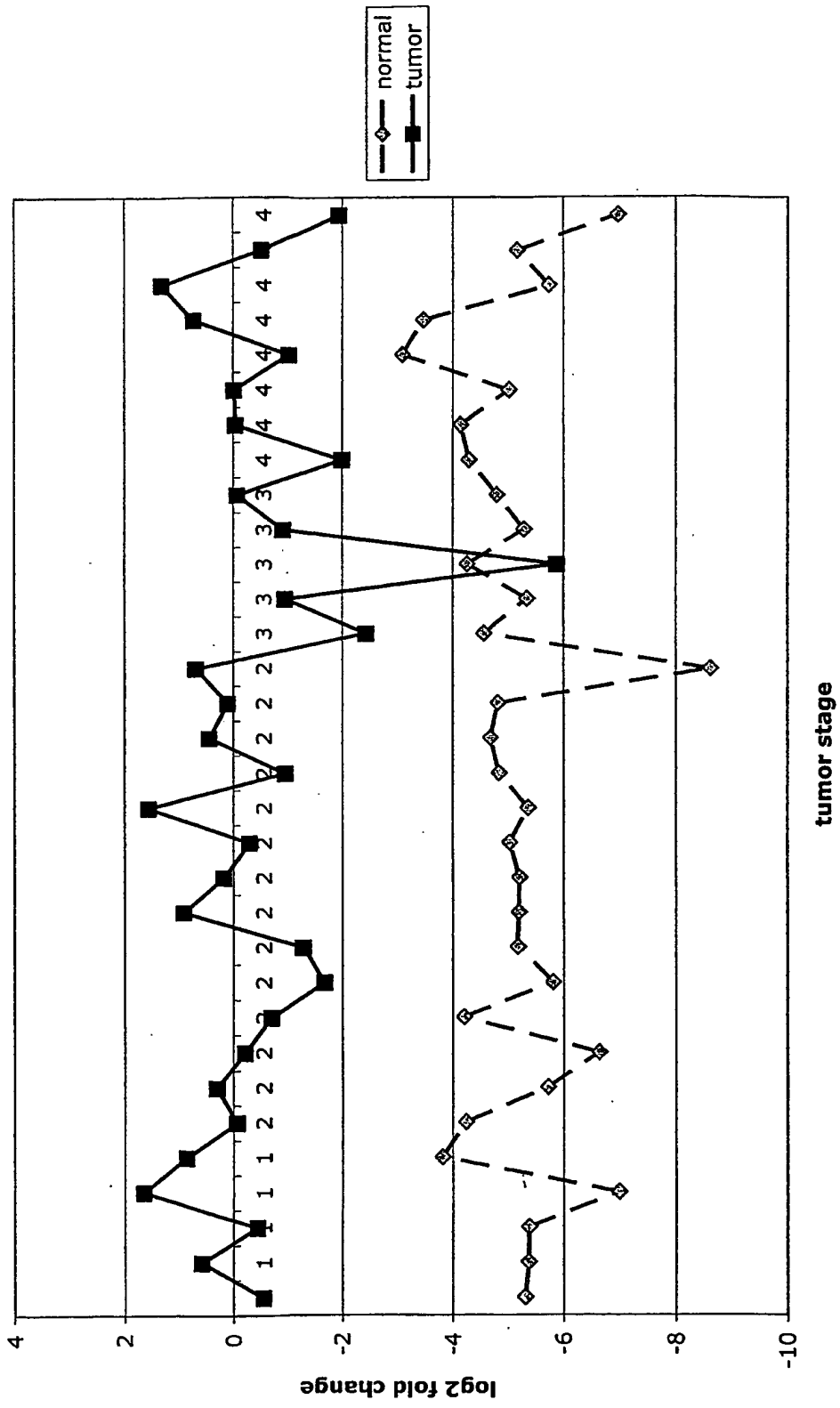


Fig. 10g INHBA



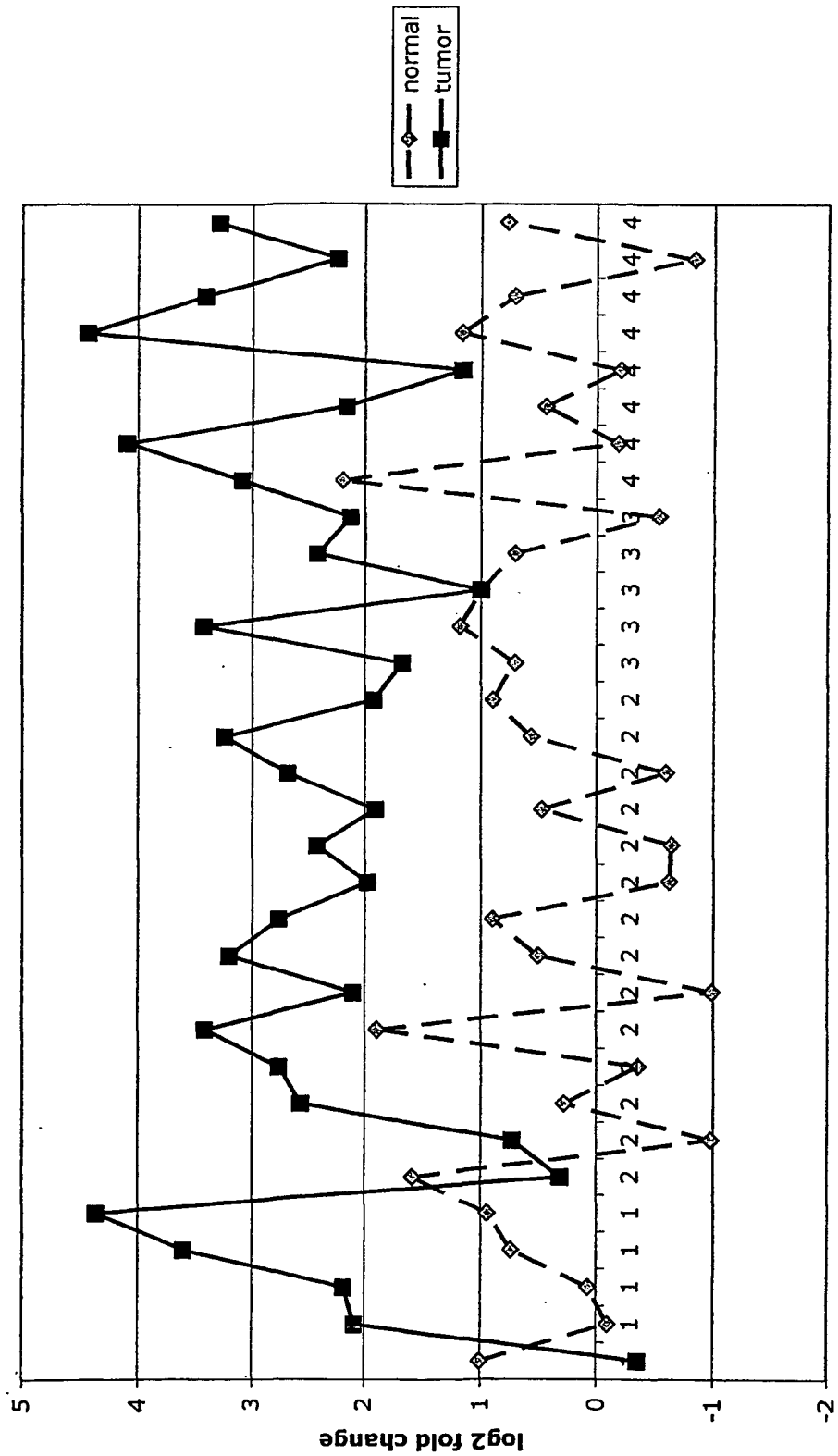
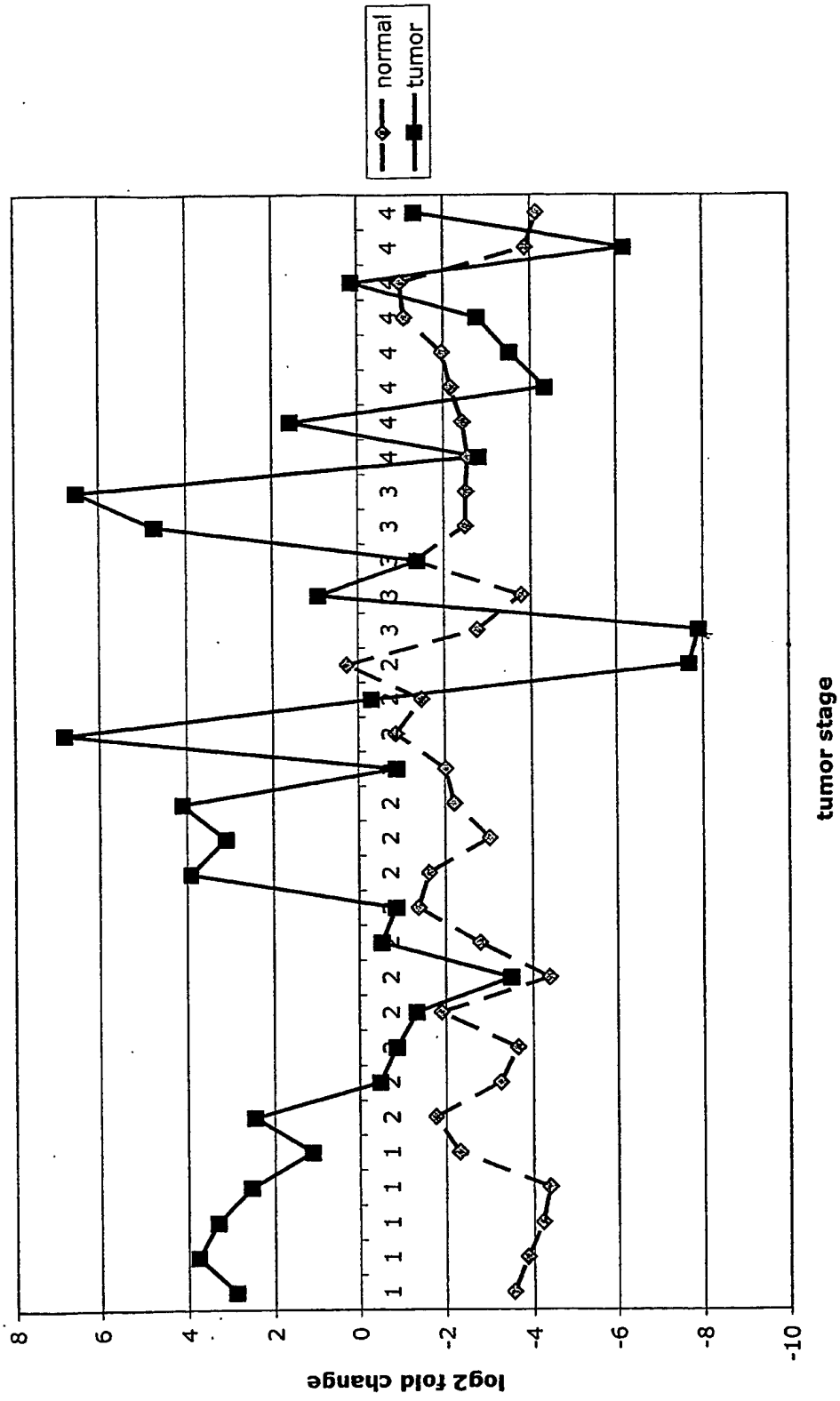


Fig. 10h IGFBP7

Fig. 10i KLK10



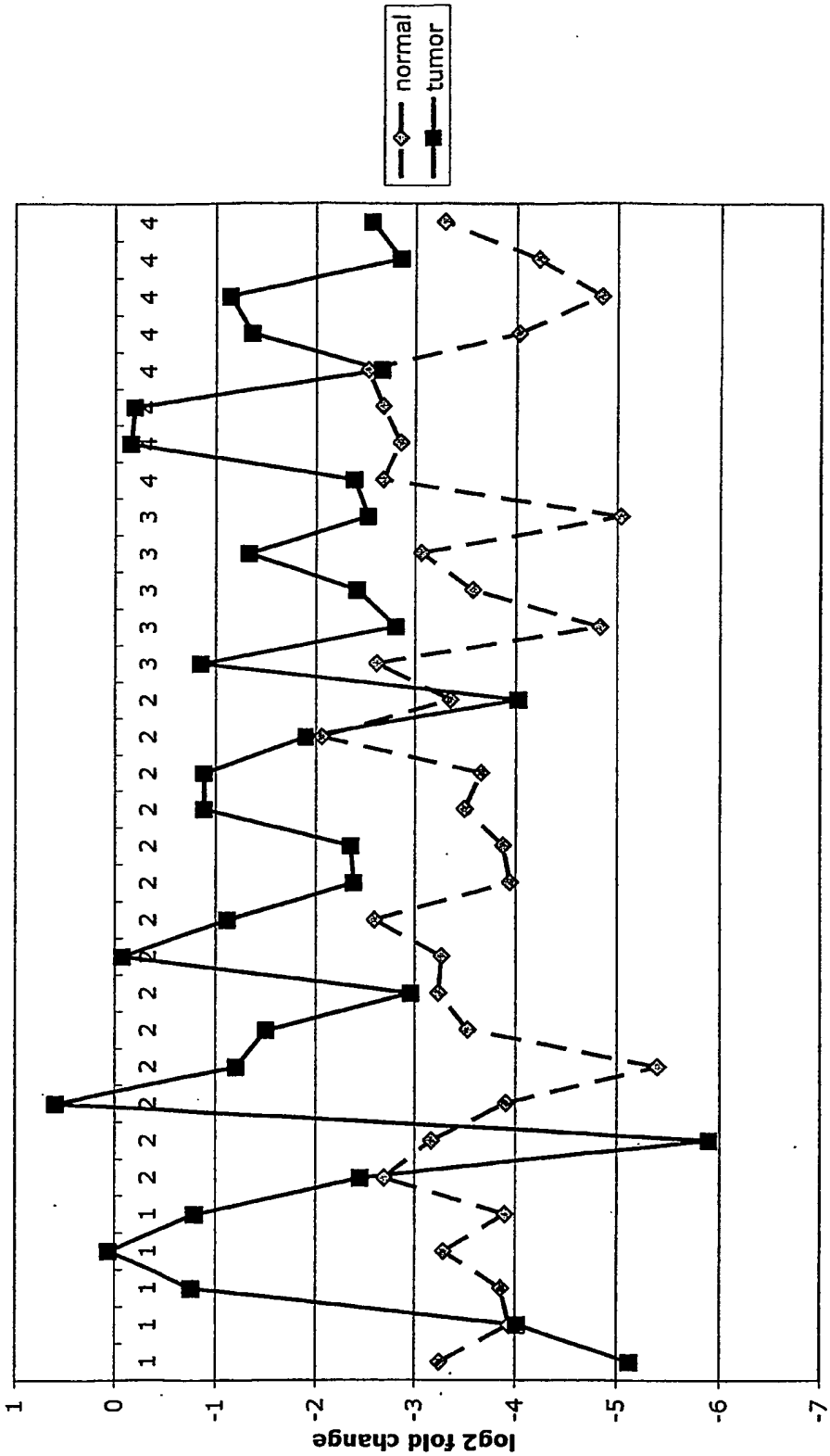


Fig. 10j LEPRE1

Fig. 10k LUM

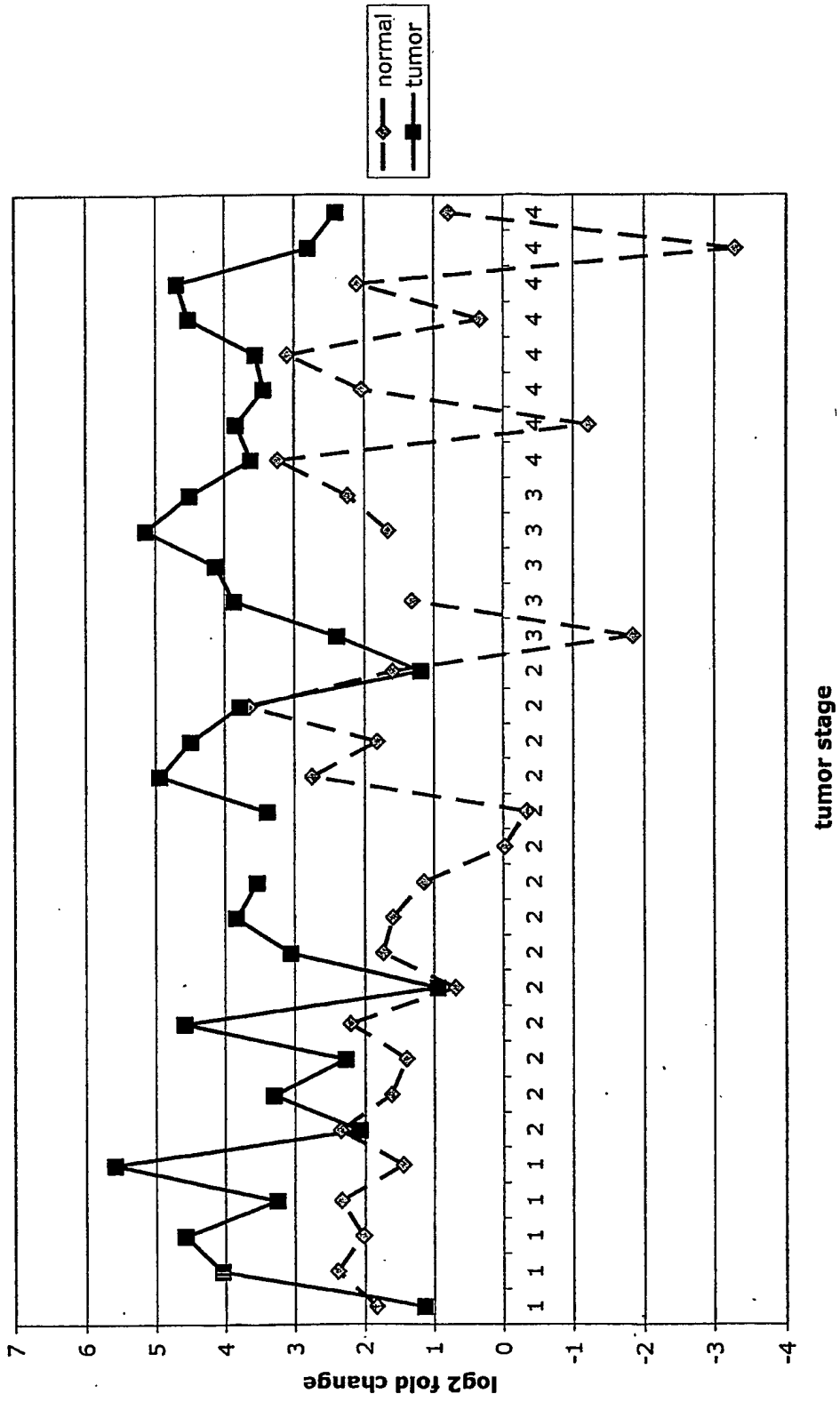
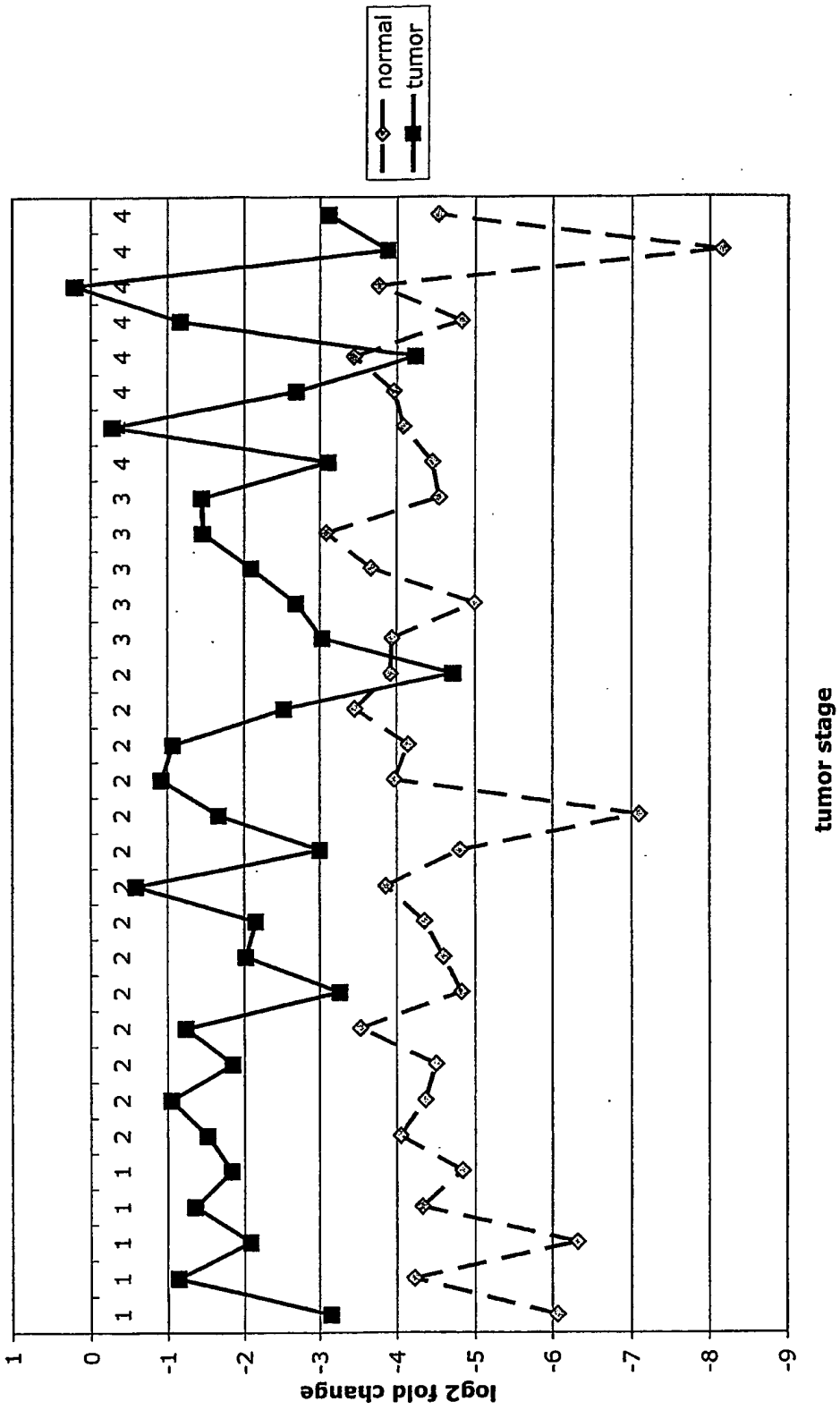

normal
tumor
log2 fold change
7
6
5
4
3
2
1
0
-1
-2
-3
-4
1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4
tumor stage

Fig. 10I LOXL2



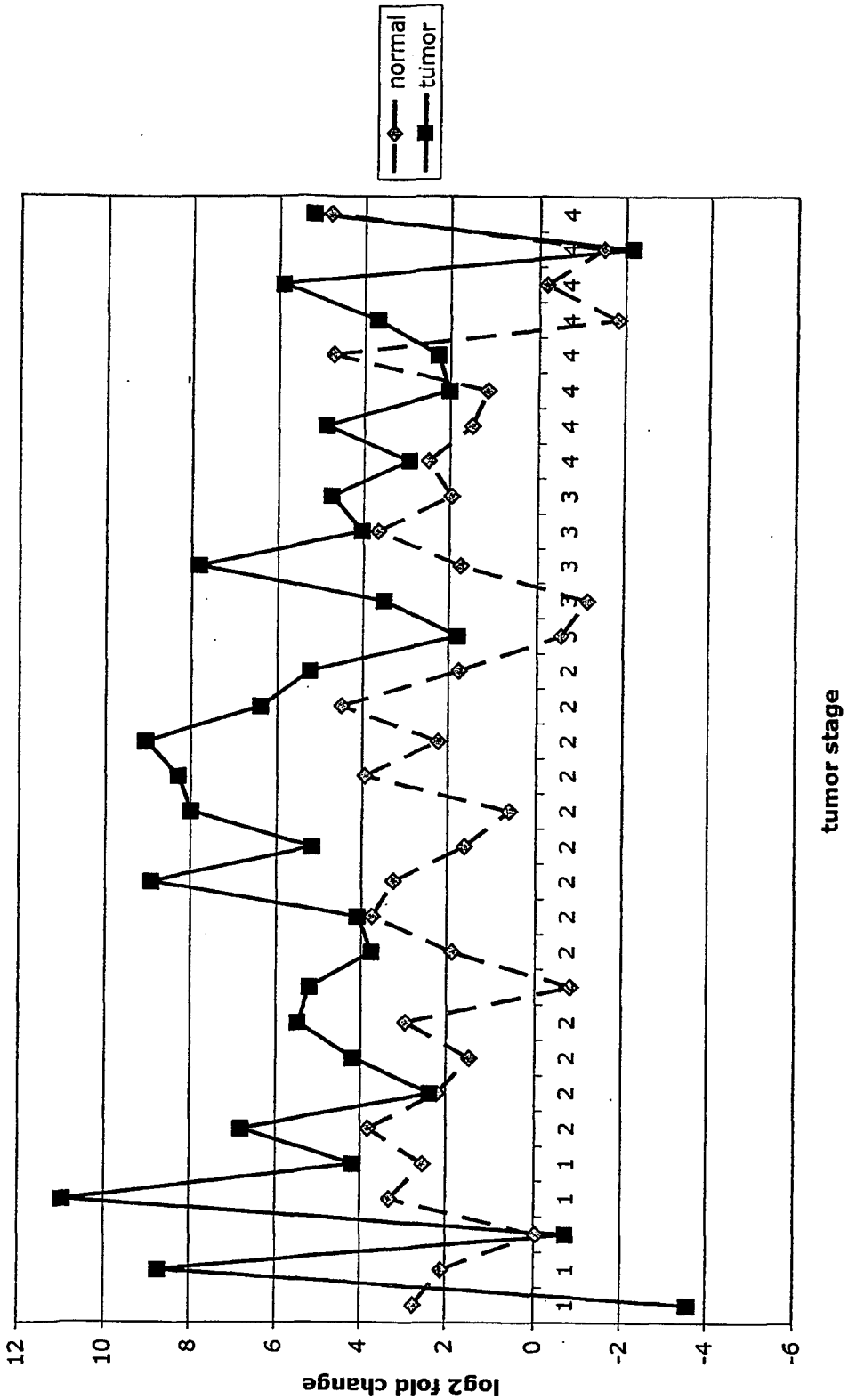


Fig. 10m MMP12

Fig.10n TIMP1

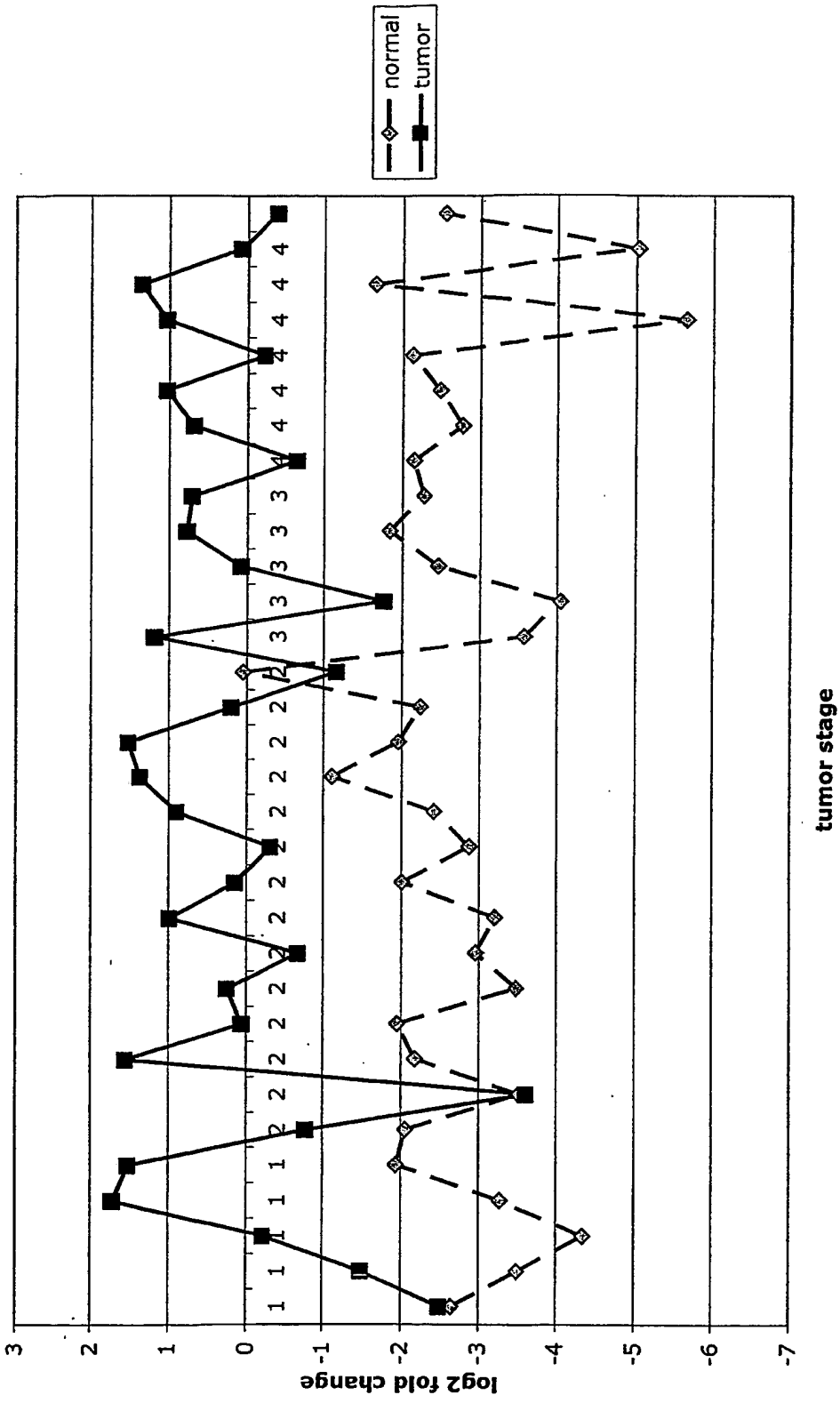
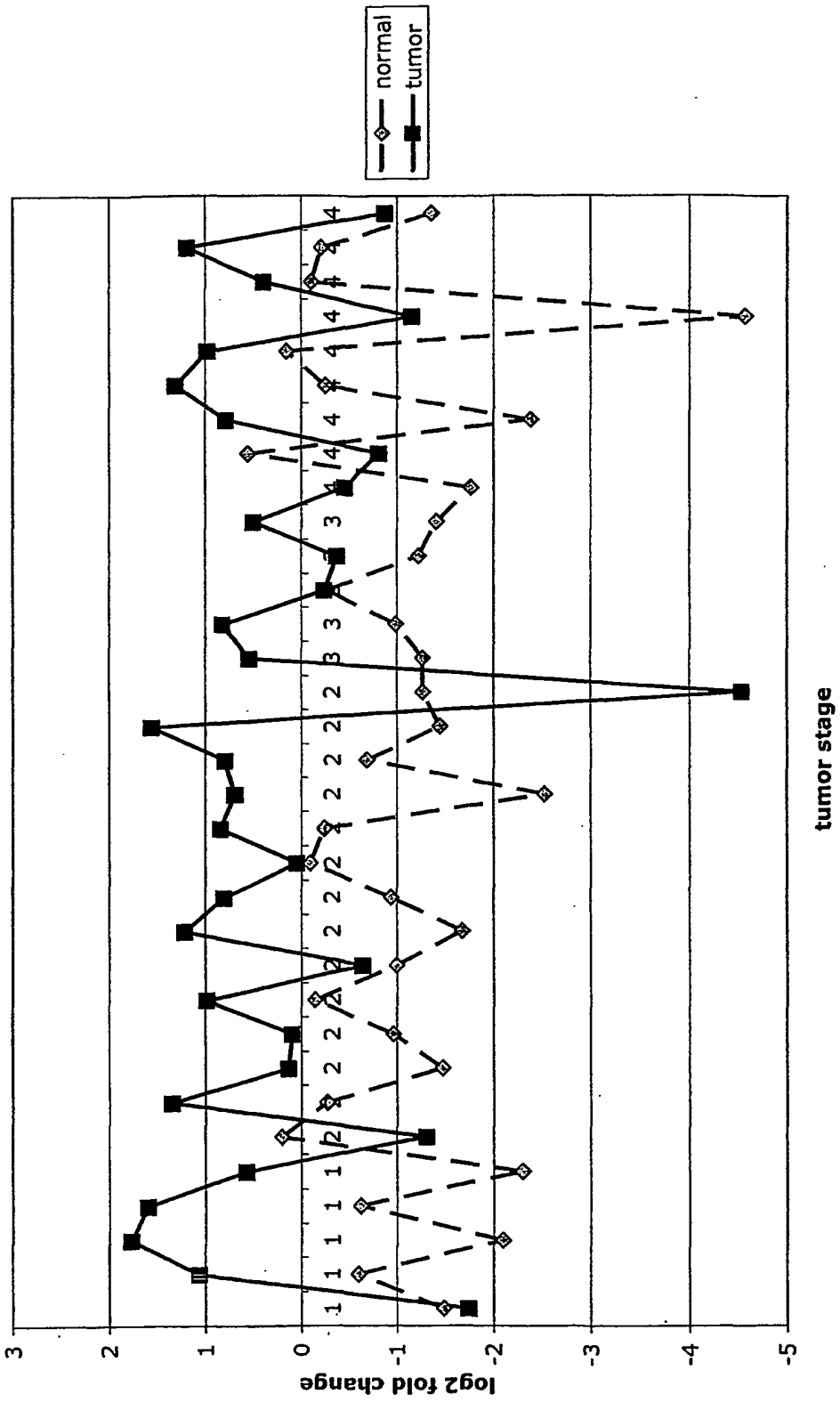


Fig. 10o ASAH1



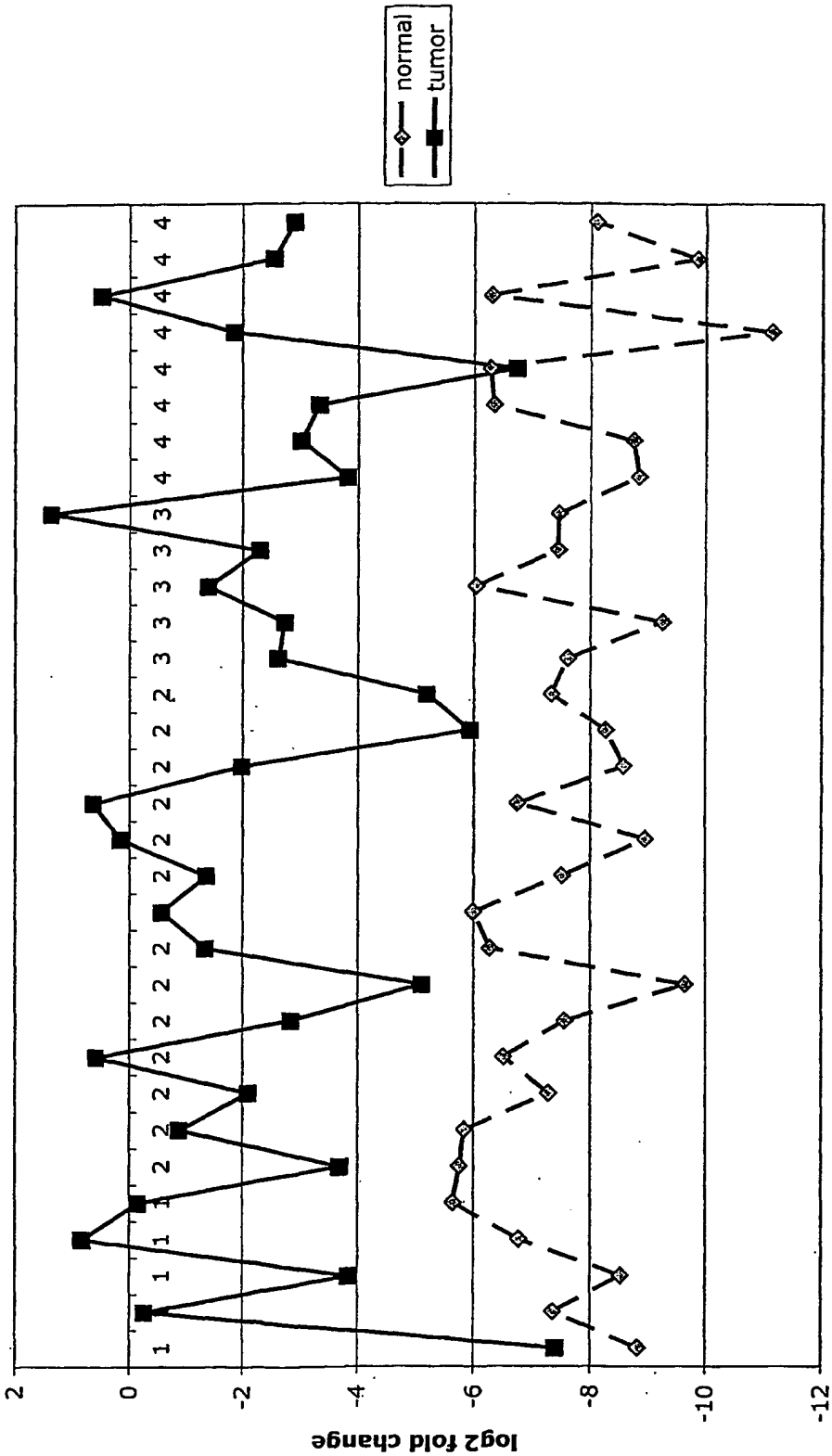
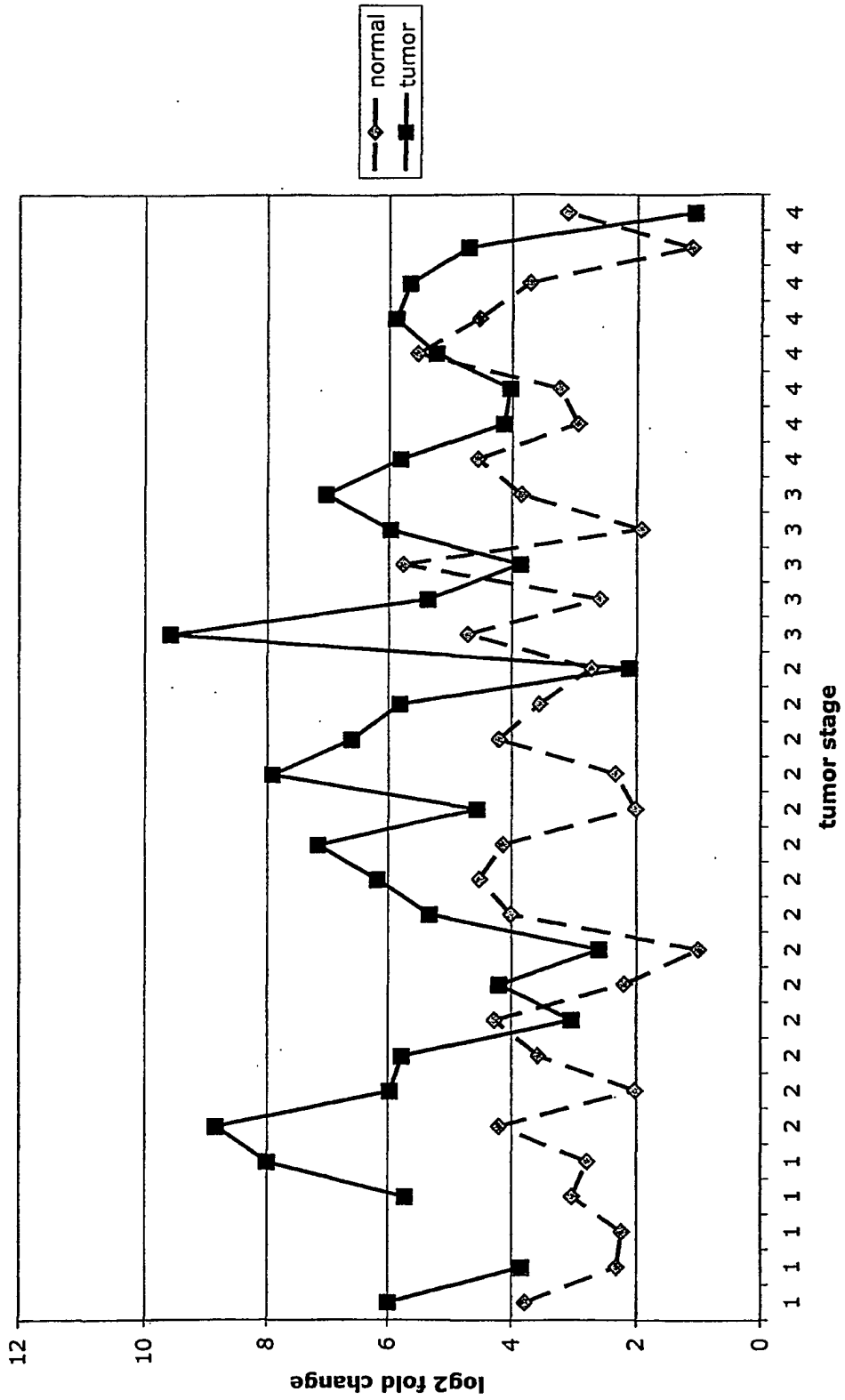


Fig. 10p SPP1

Fig. 10q SFRP2



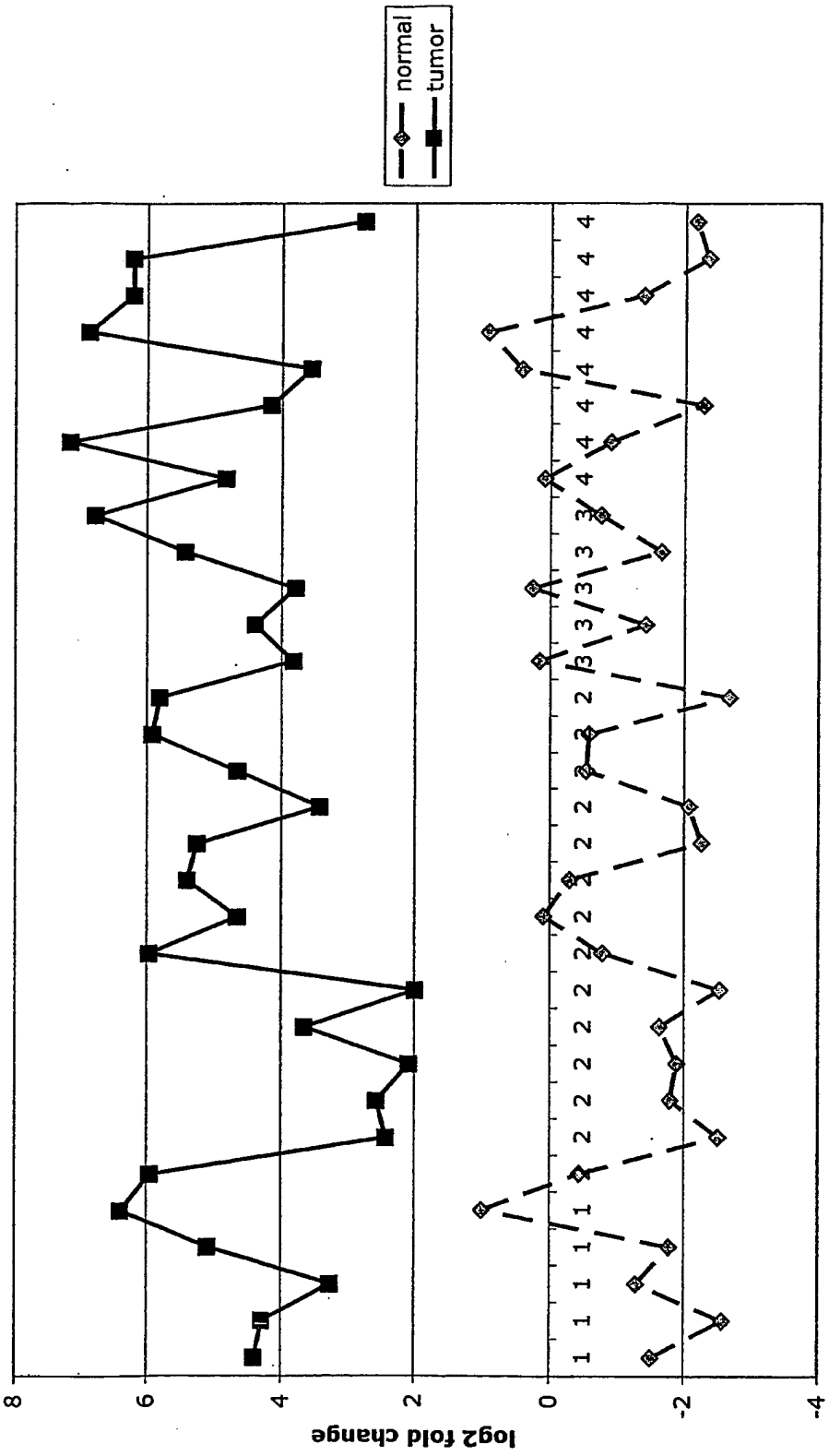
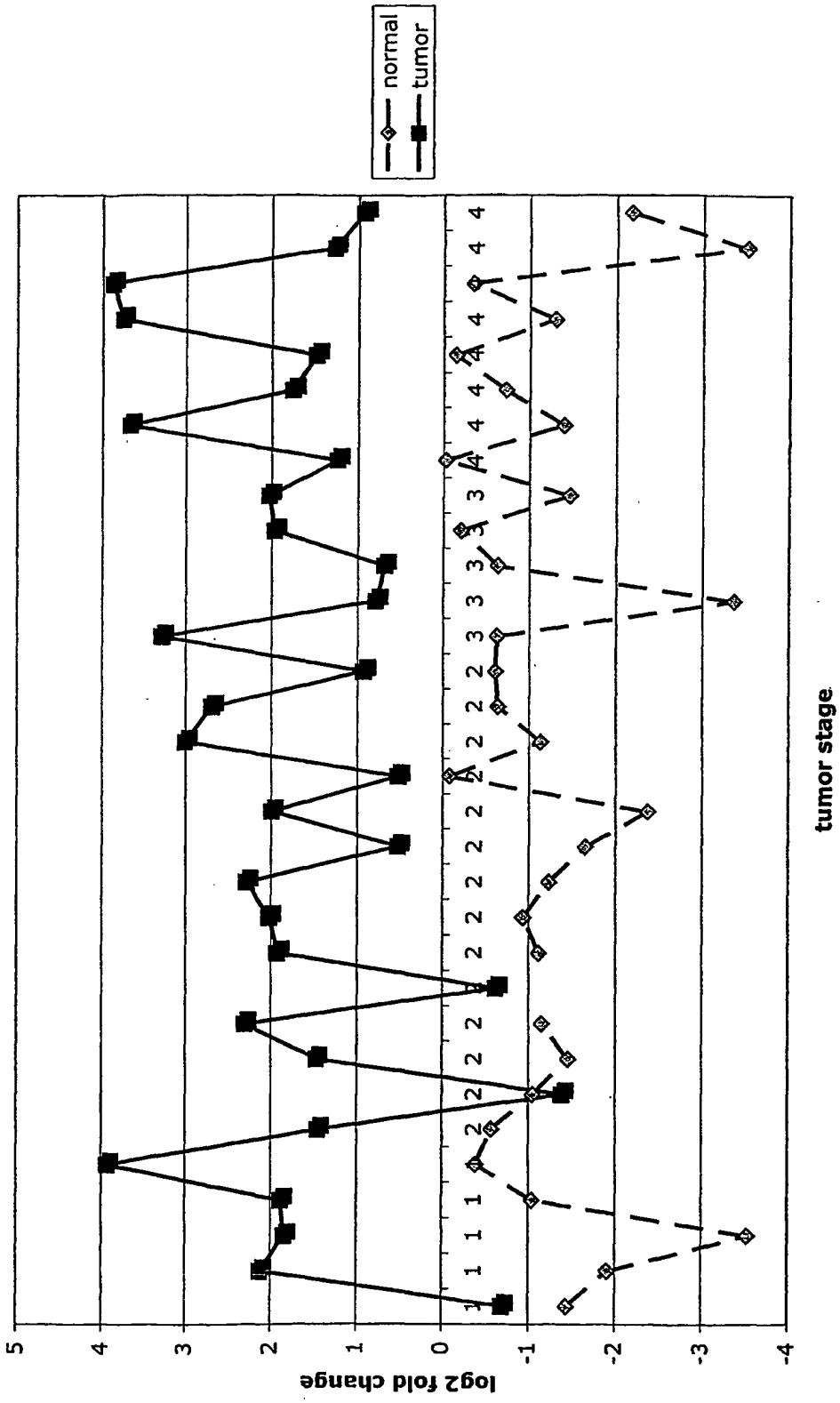


Fig. 10r SFRP4

Fig. 10s SPARC



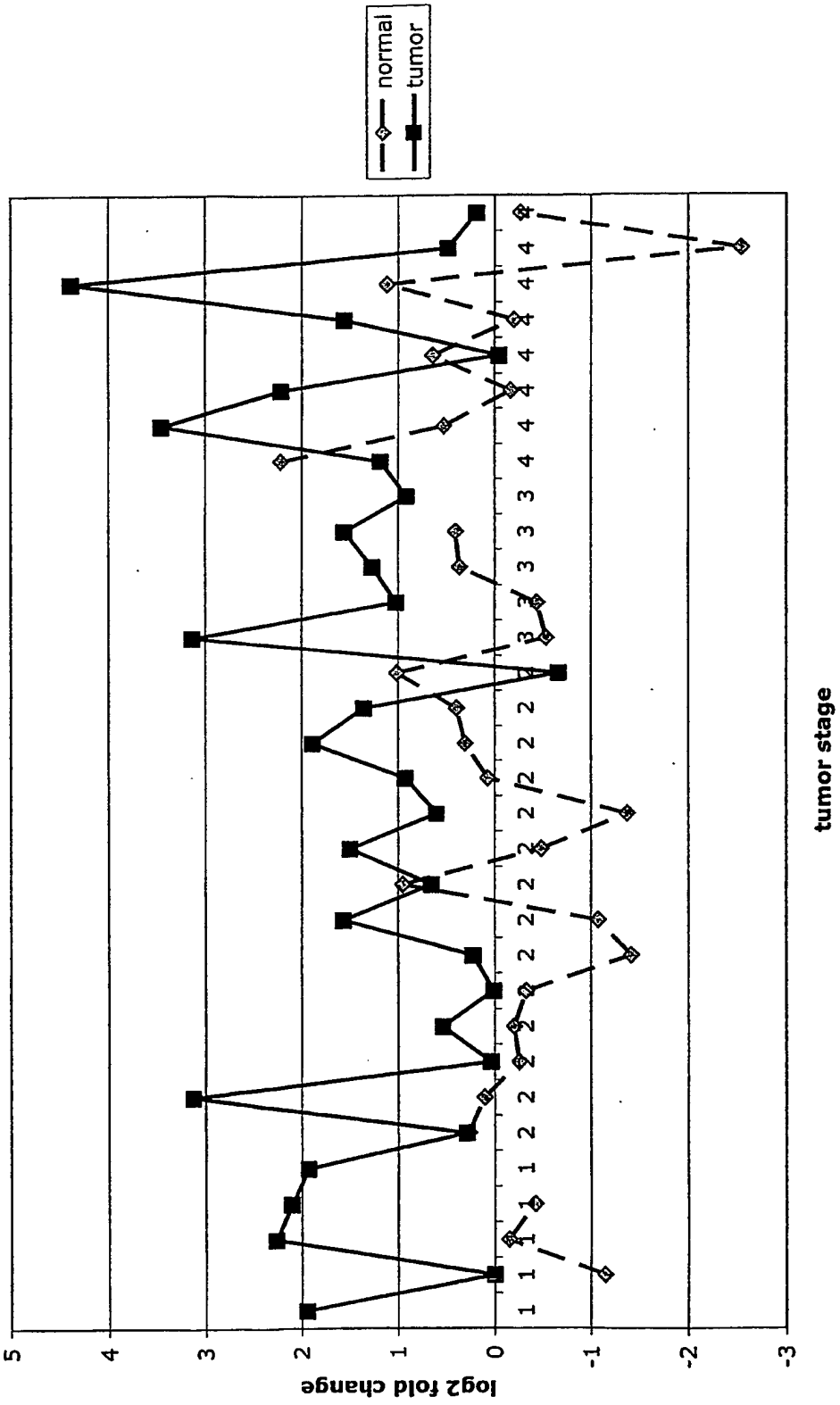


Fig. 10t PRSS11

Fig. 10u THBS2

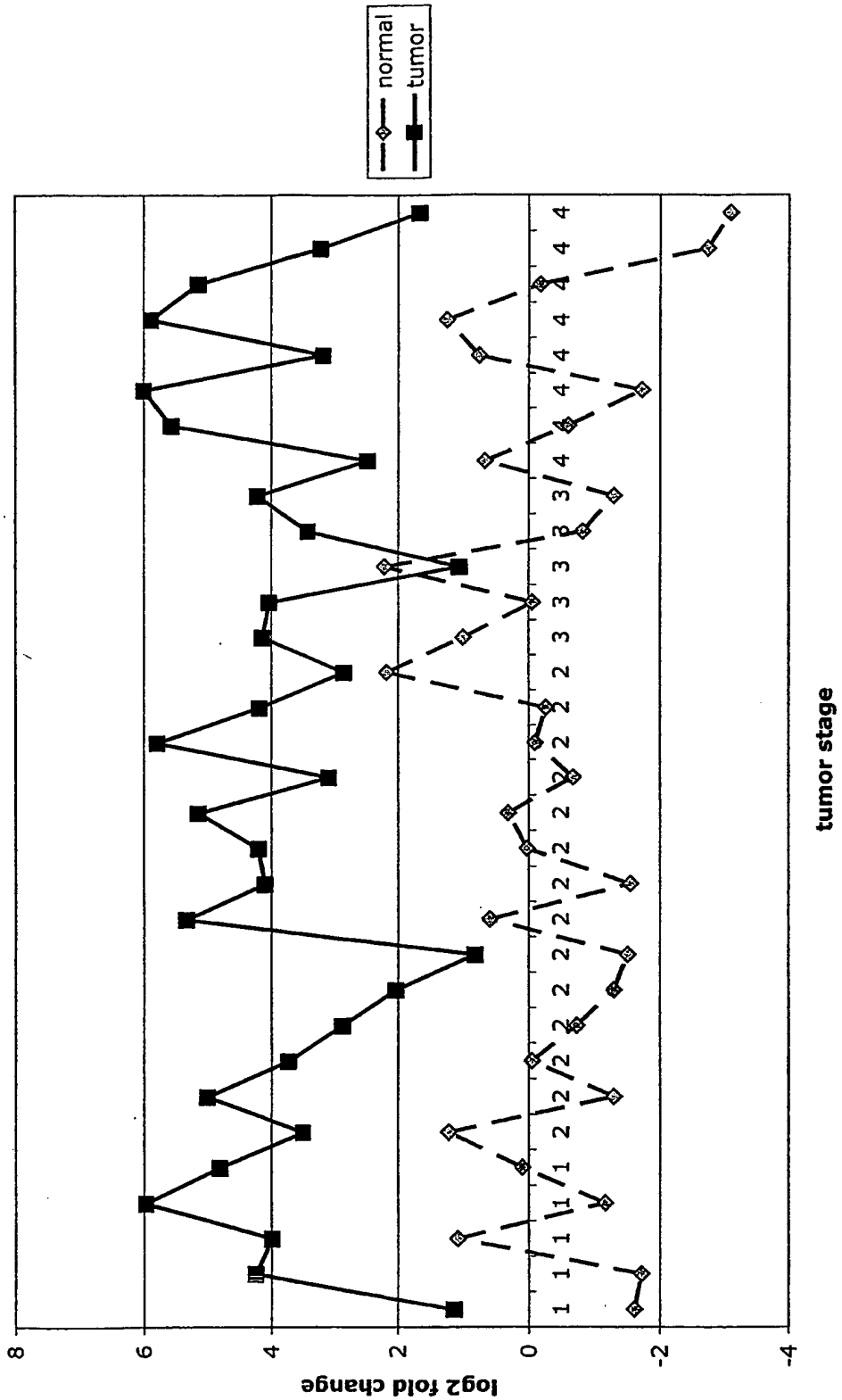


Fig. 10v TG

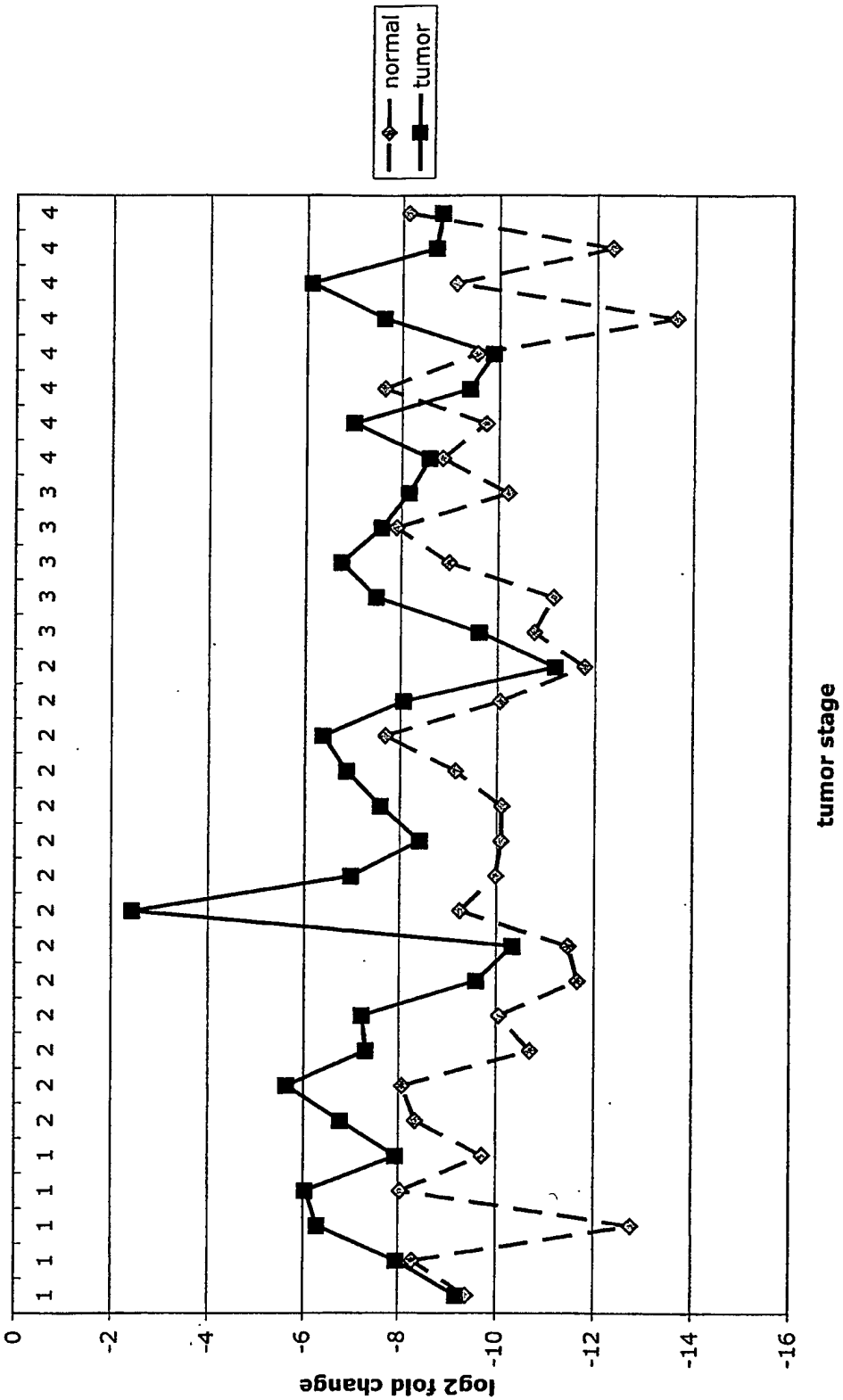


Fig. 10w TGFBI

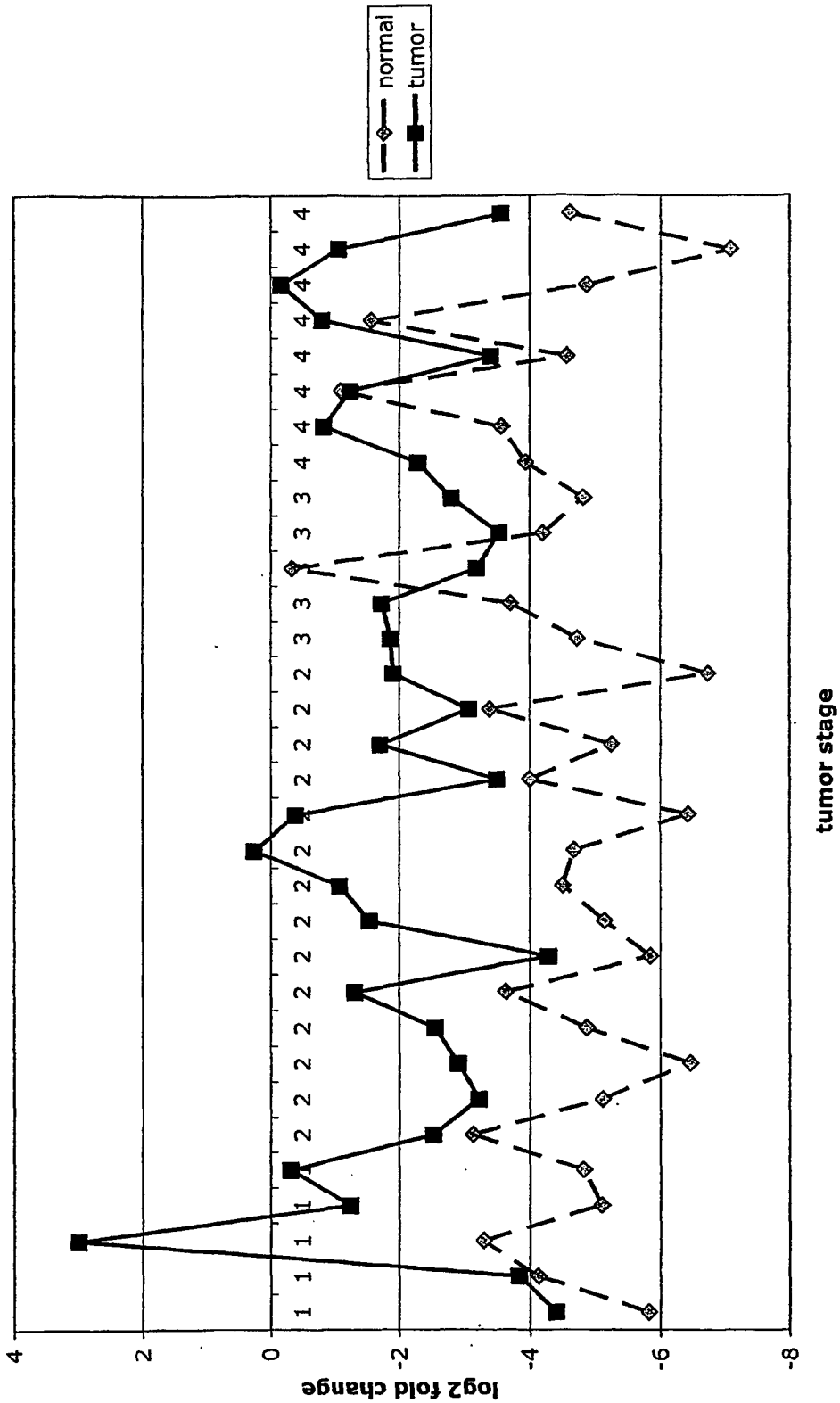


Fig. 10x CGR11

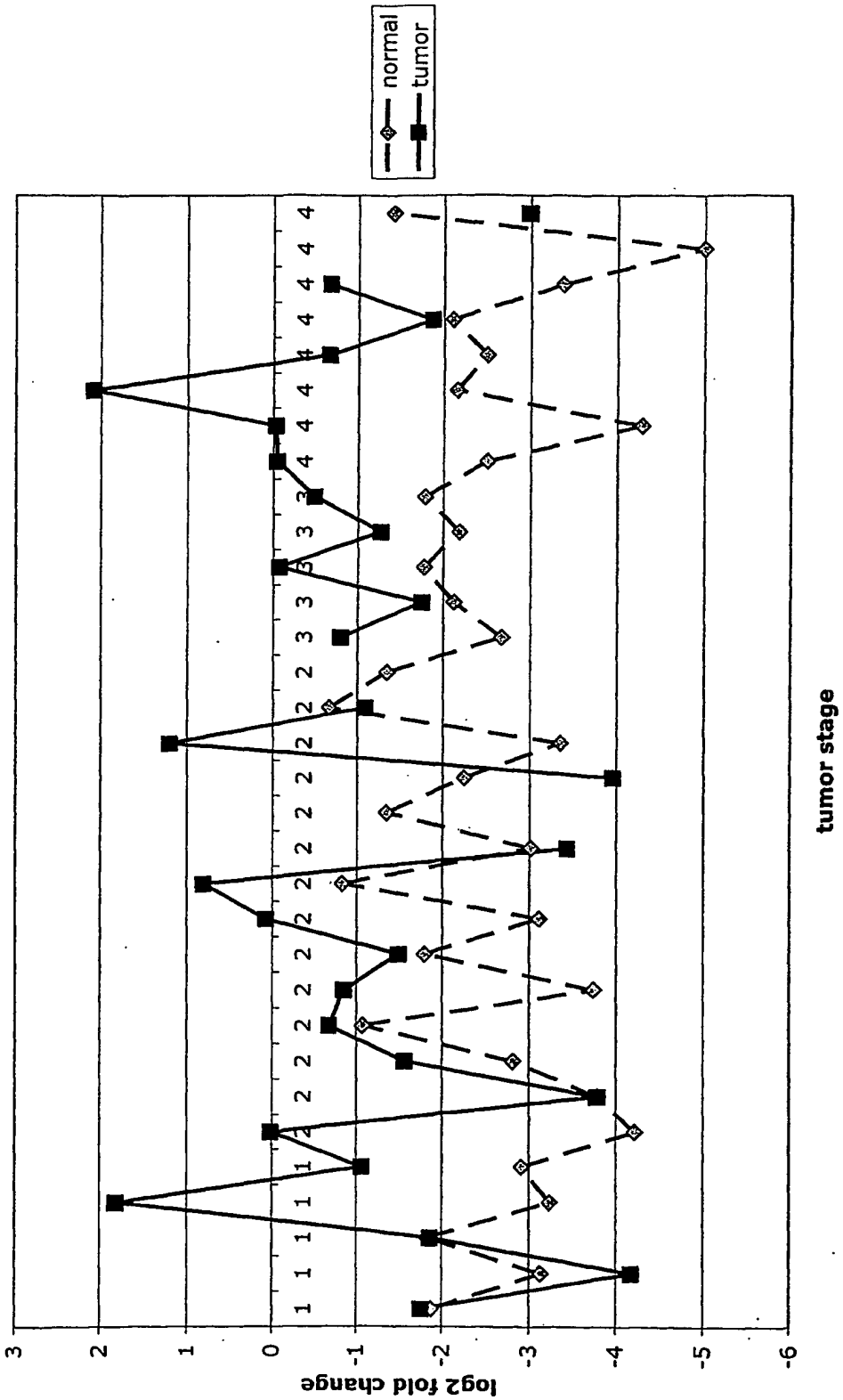
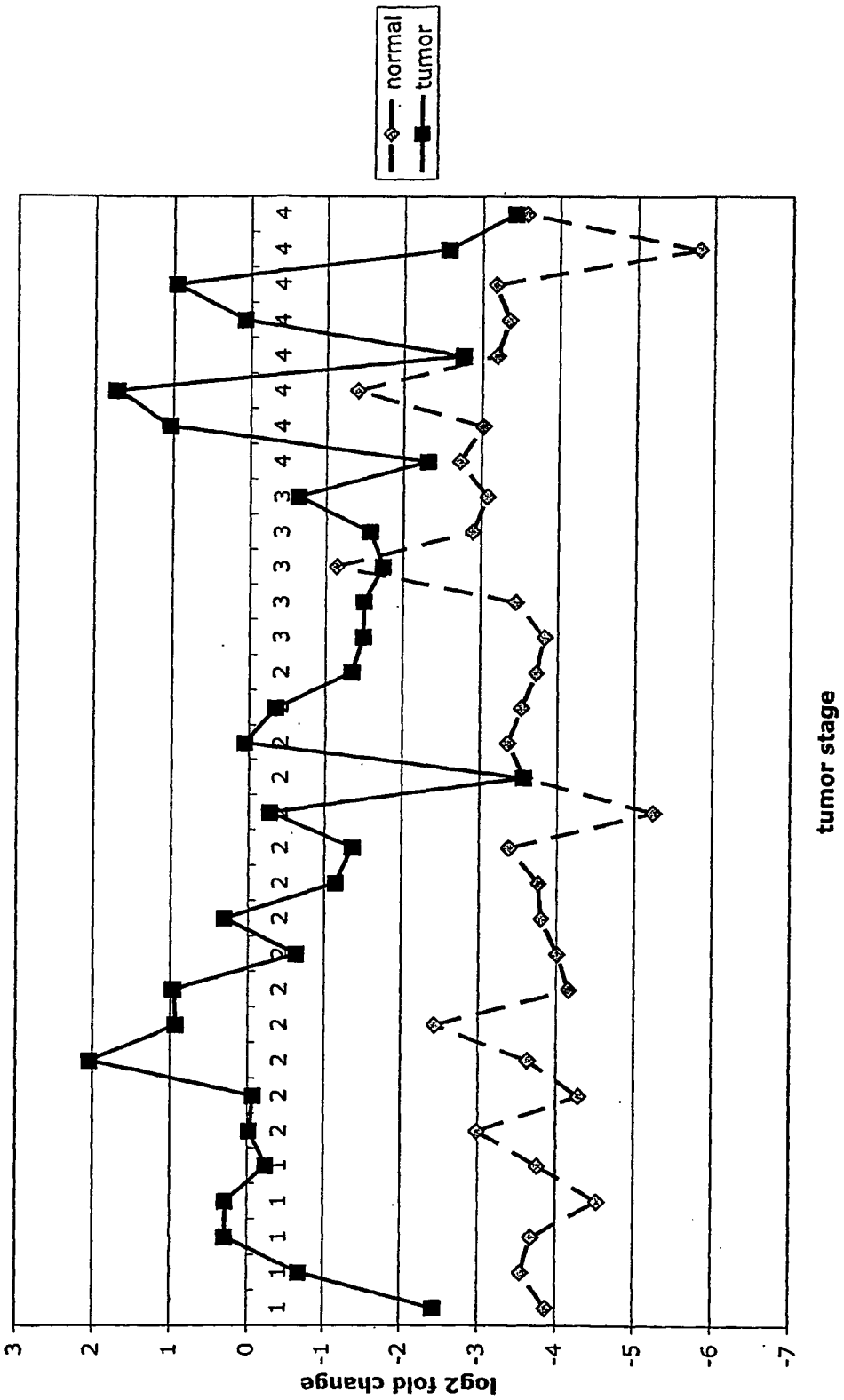


Fig. 10y SERPINH1



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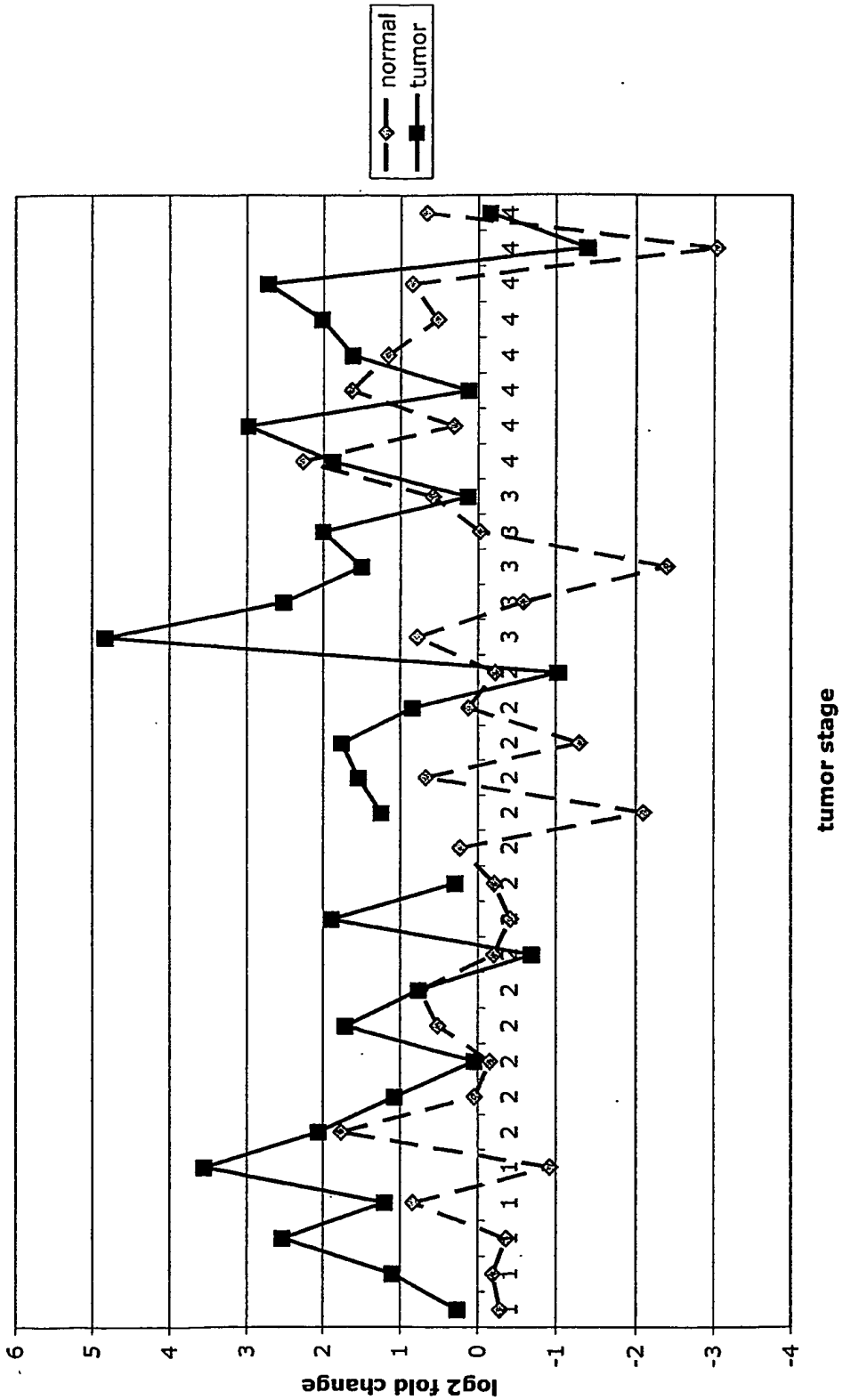

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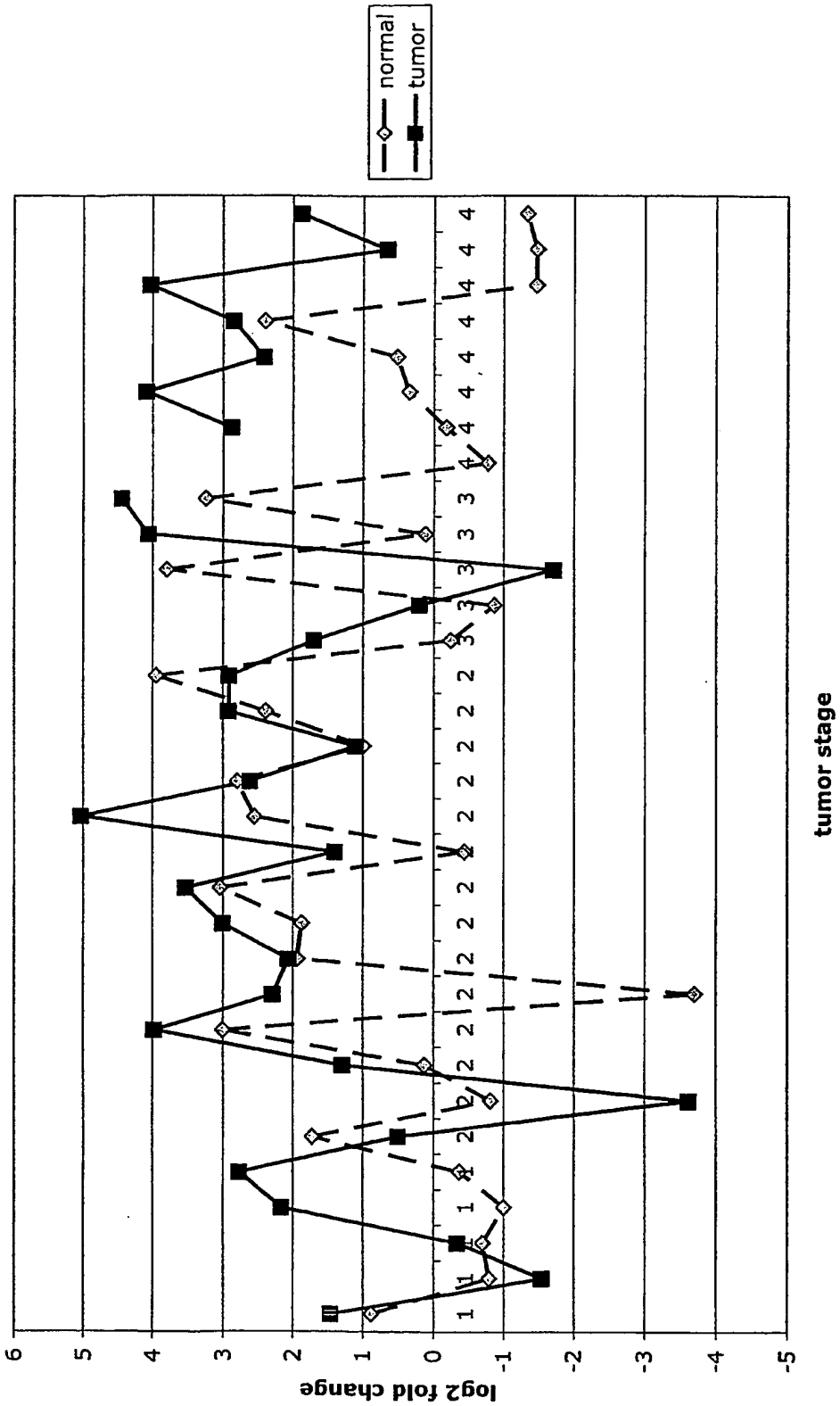

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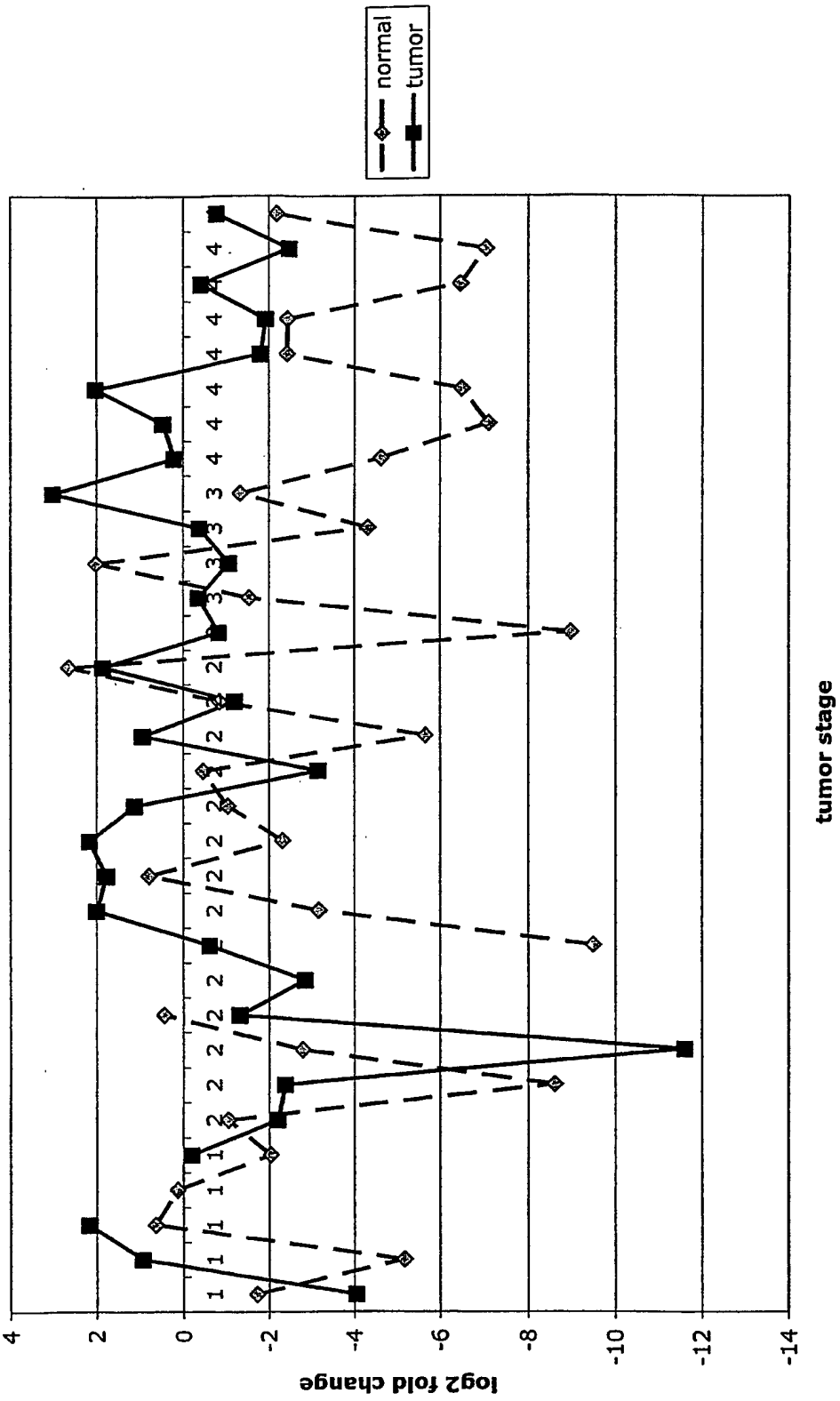

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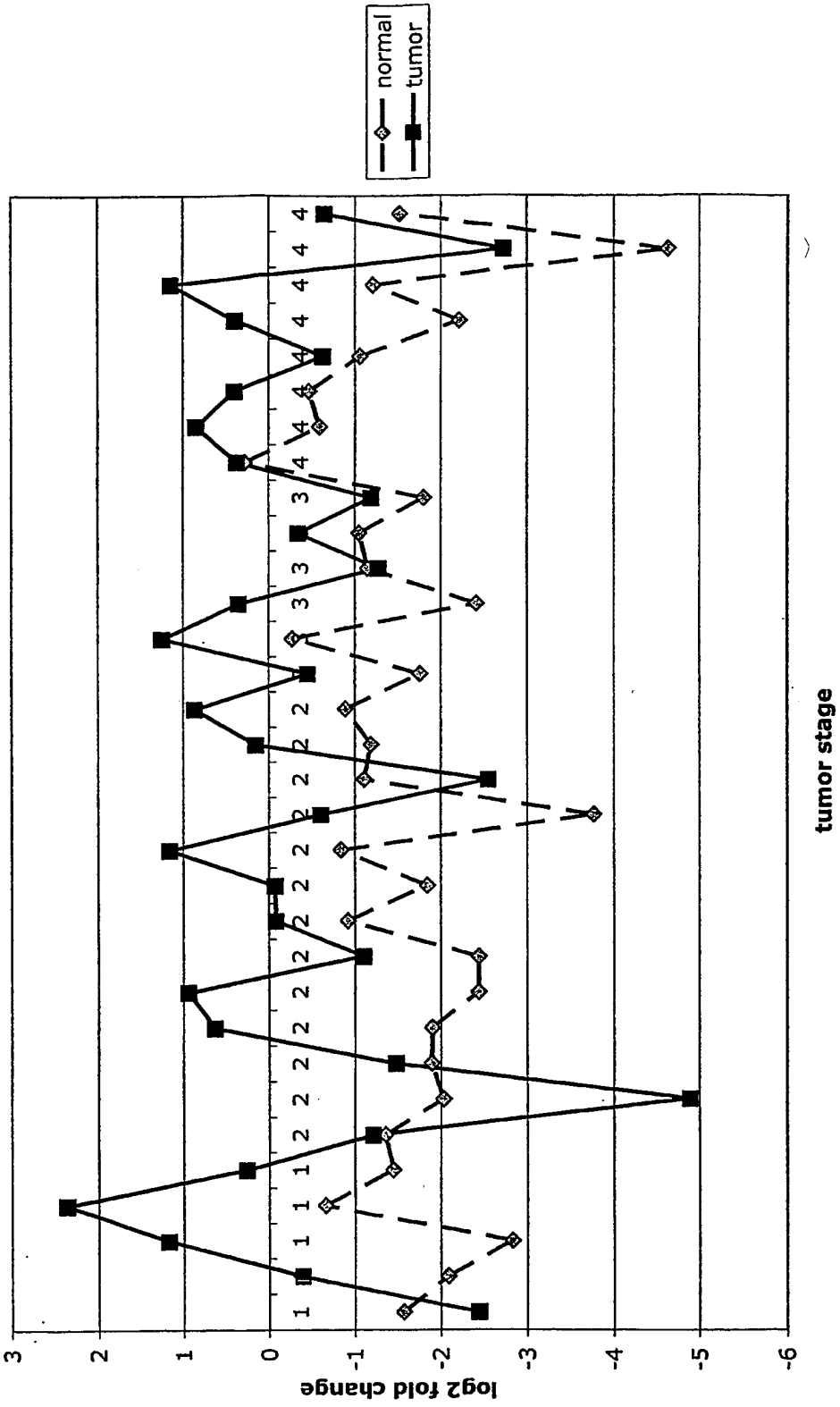

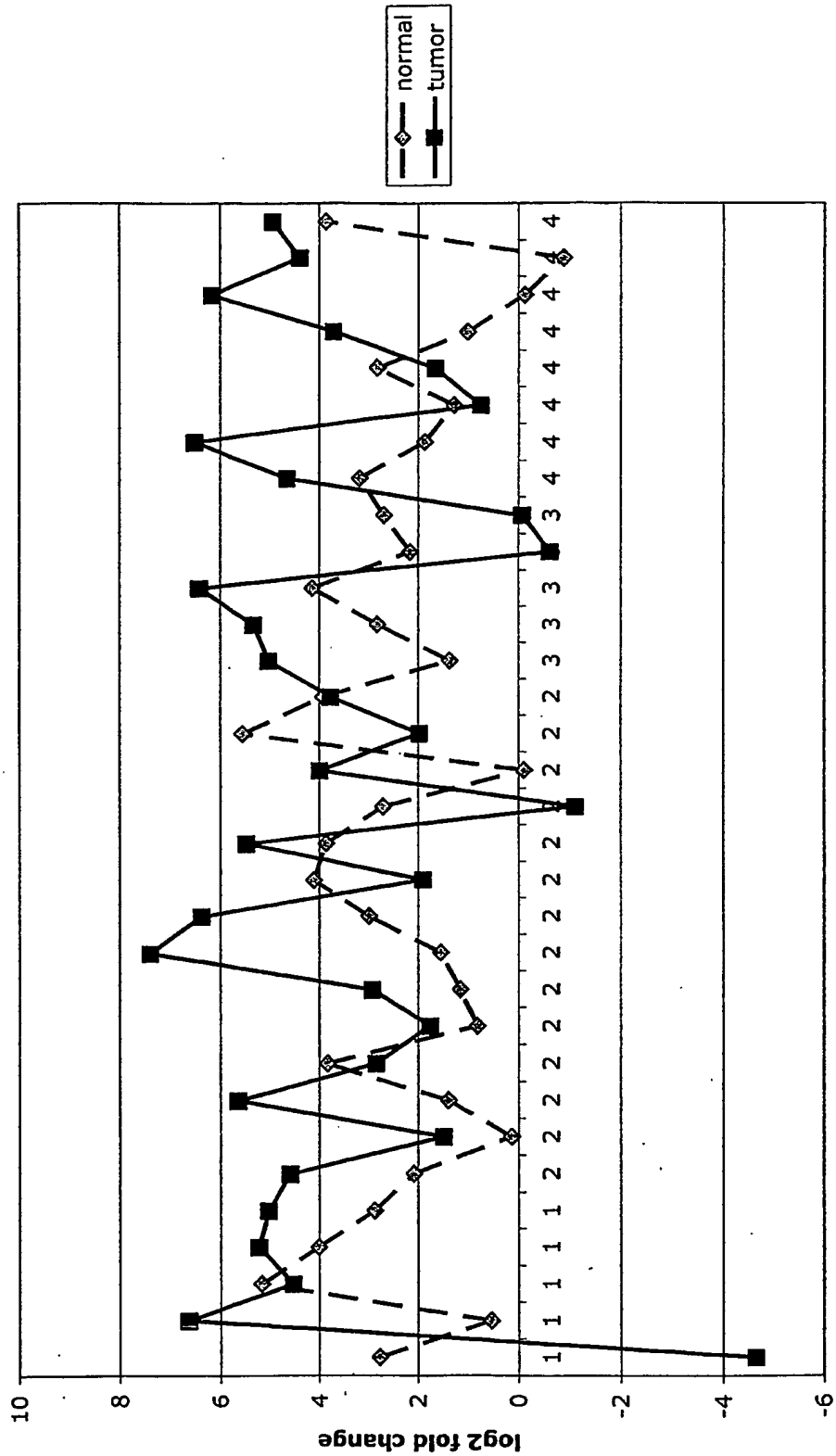
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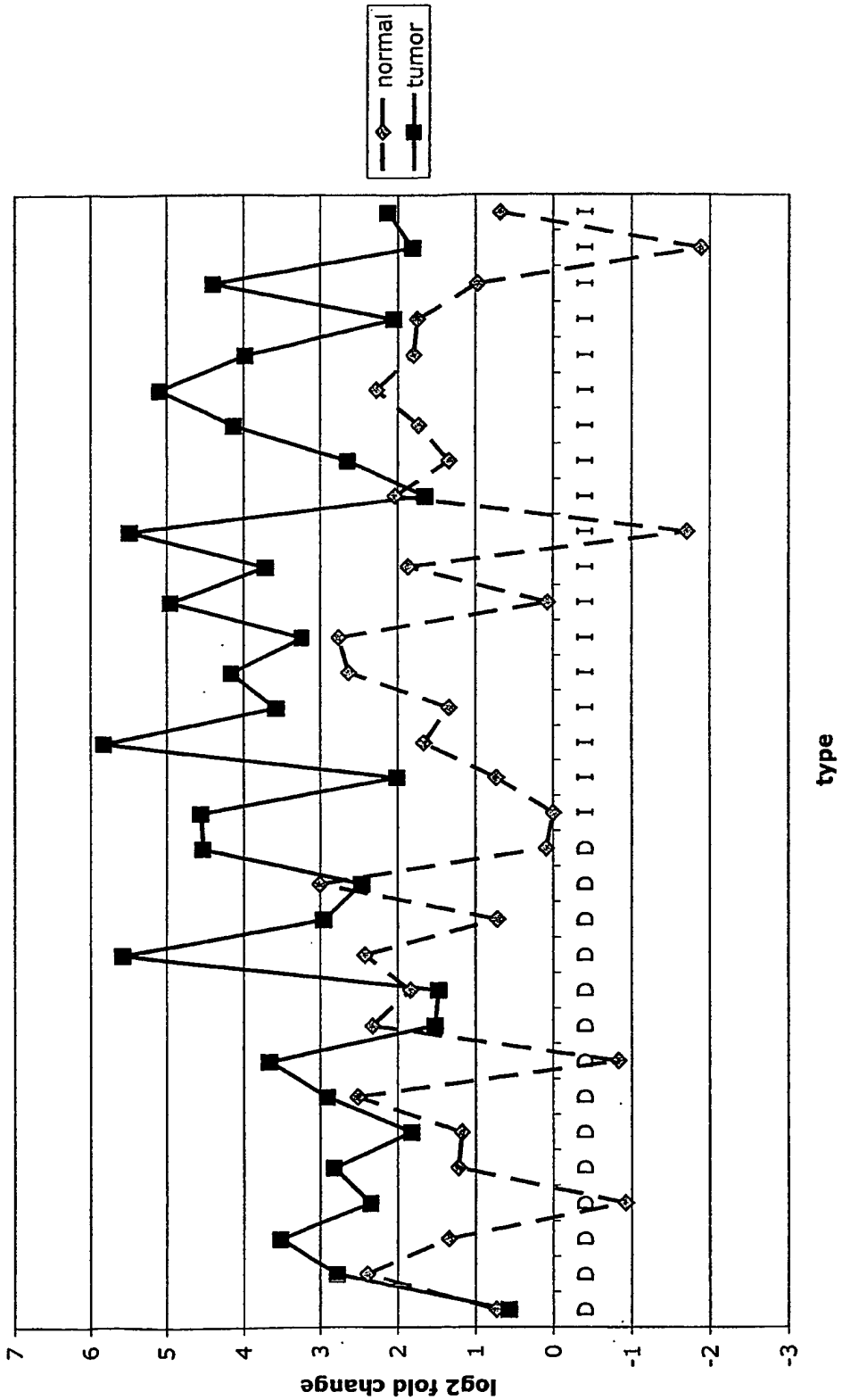

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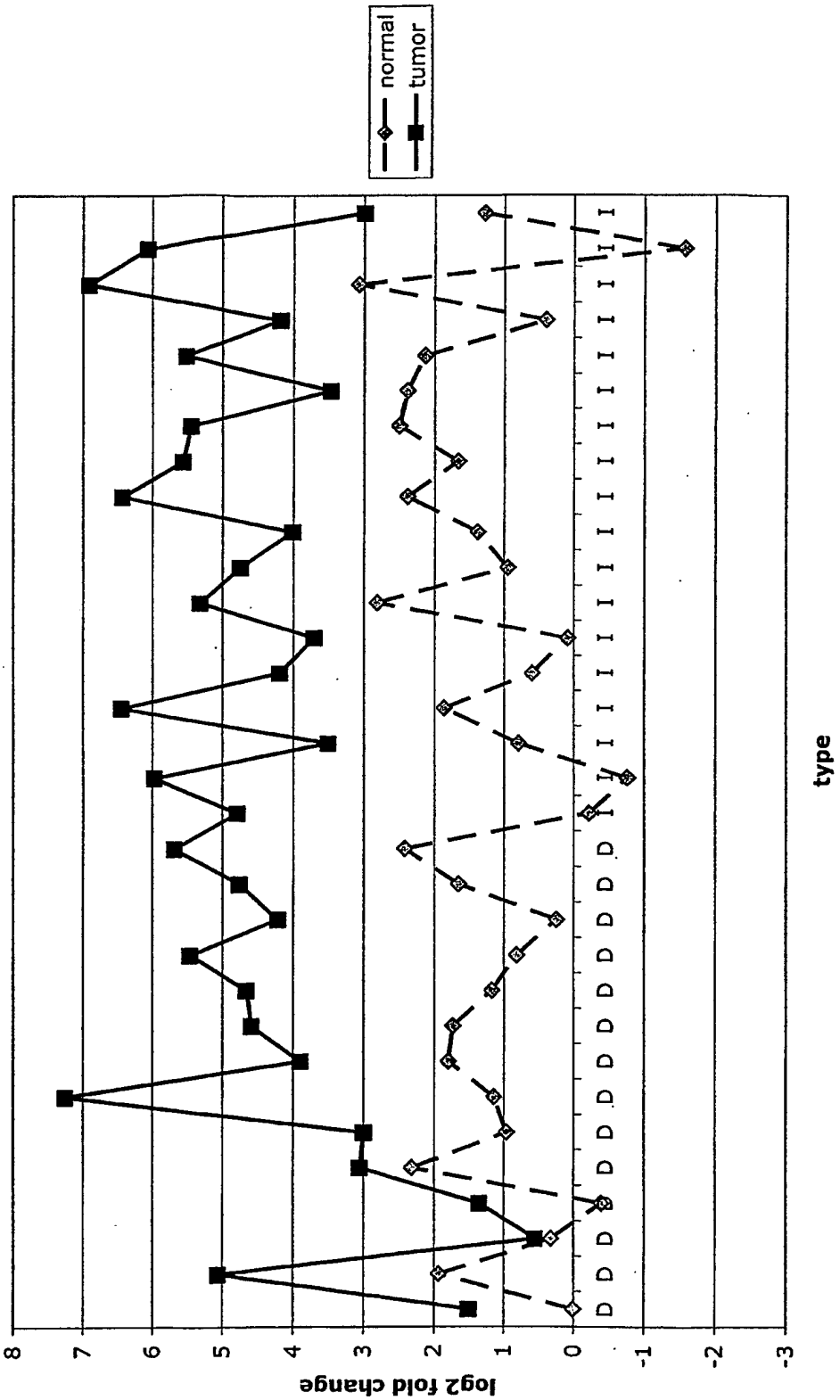

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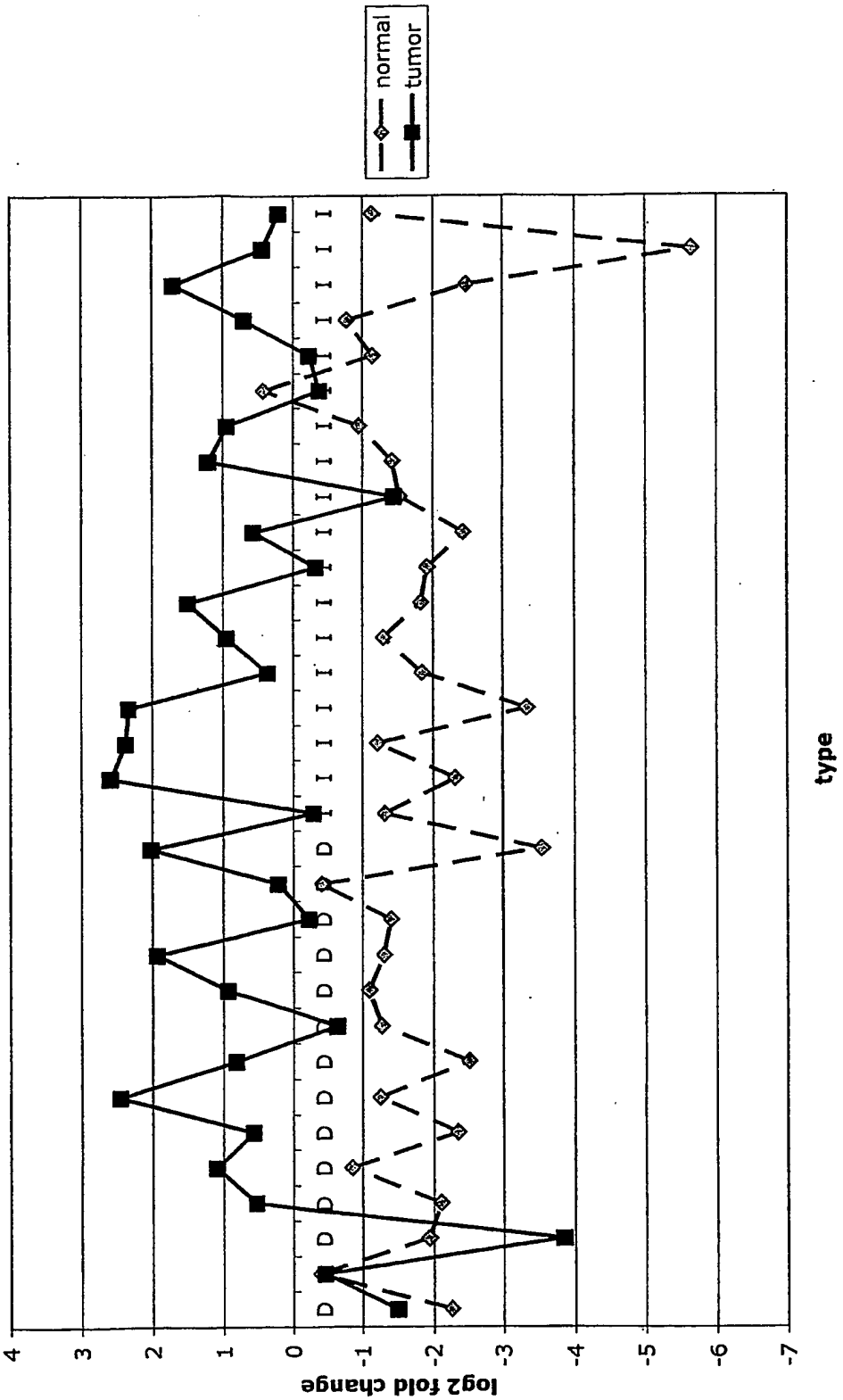

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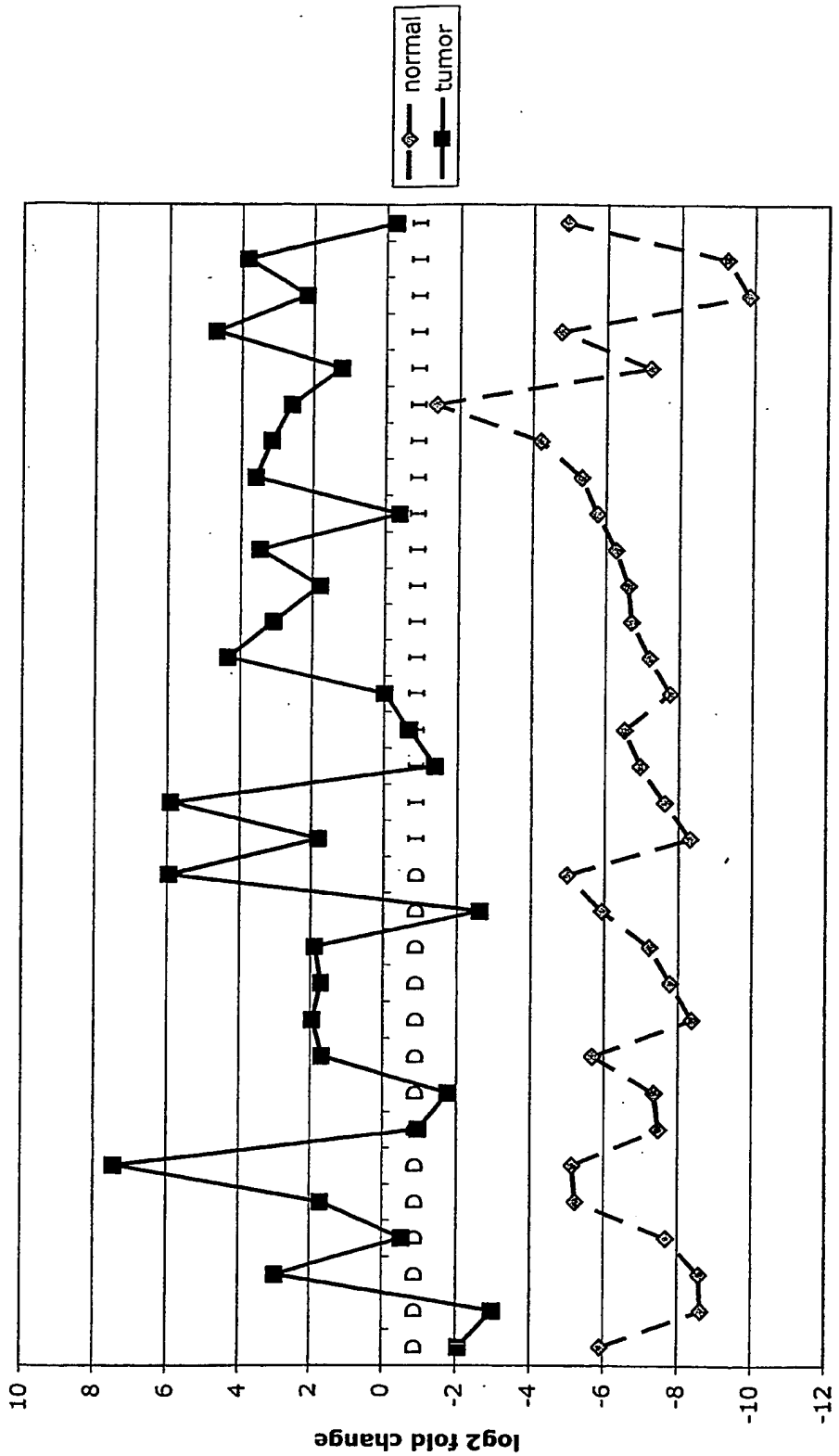

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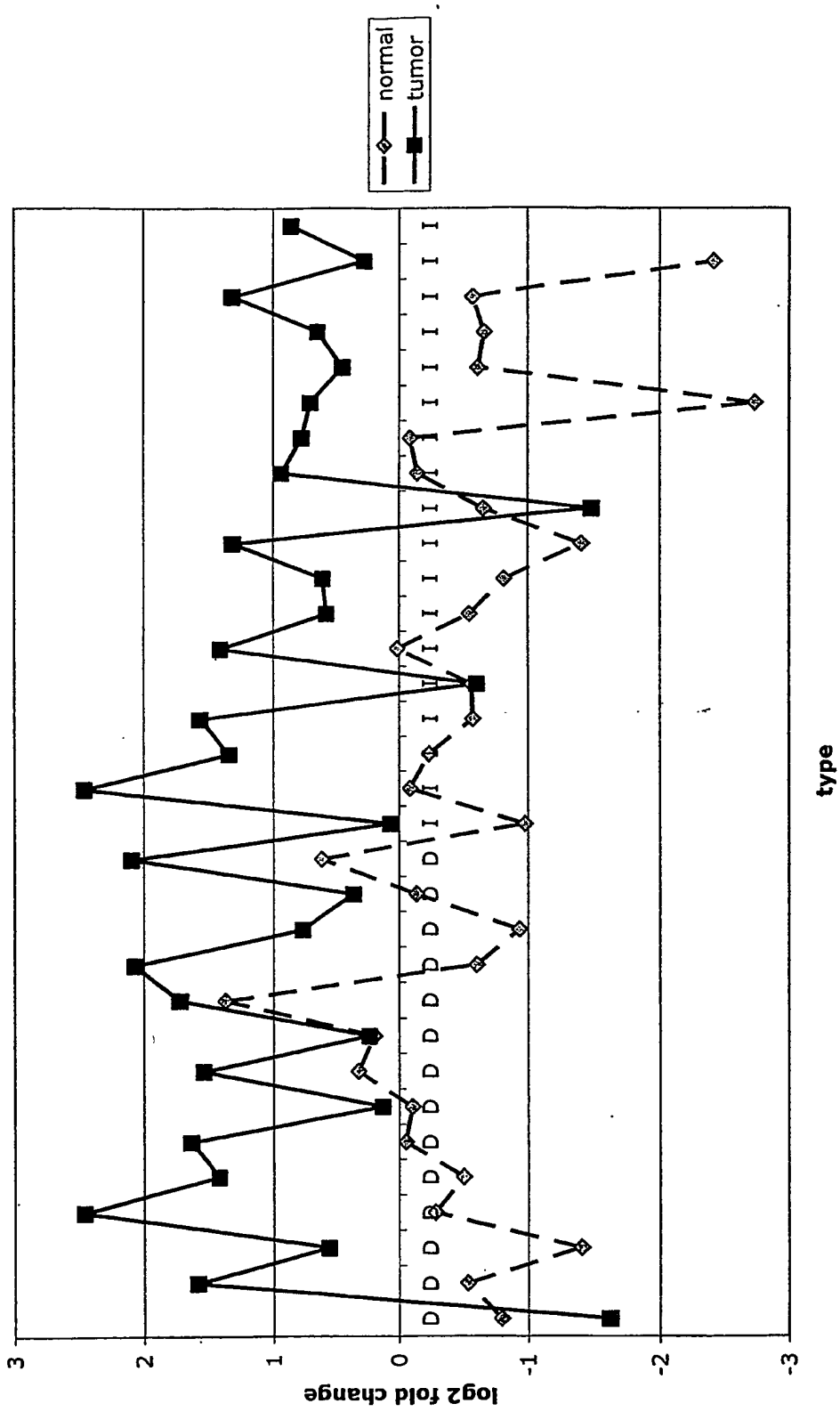

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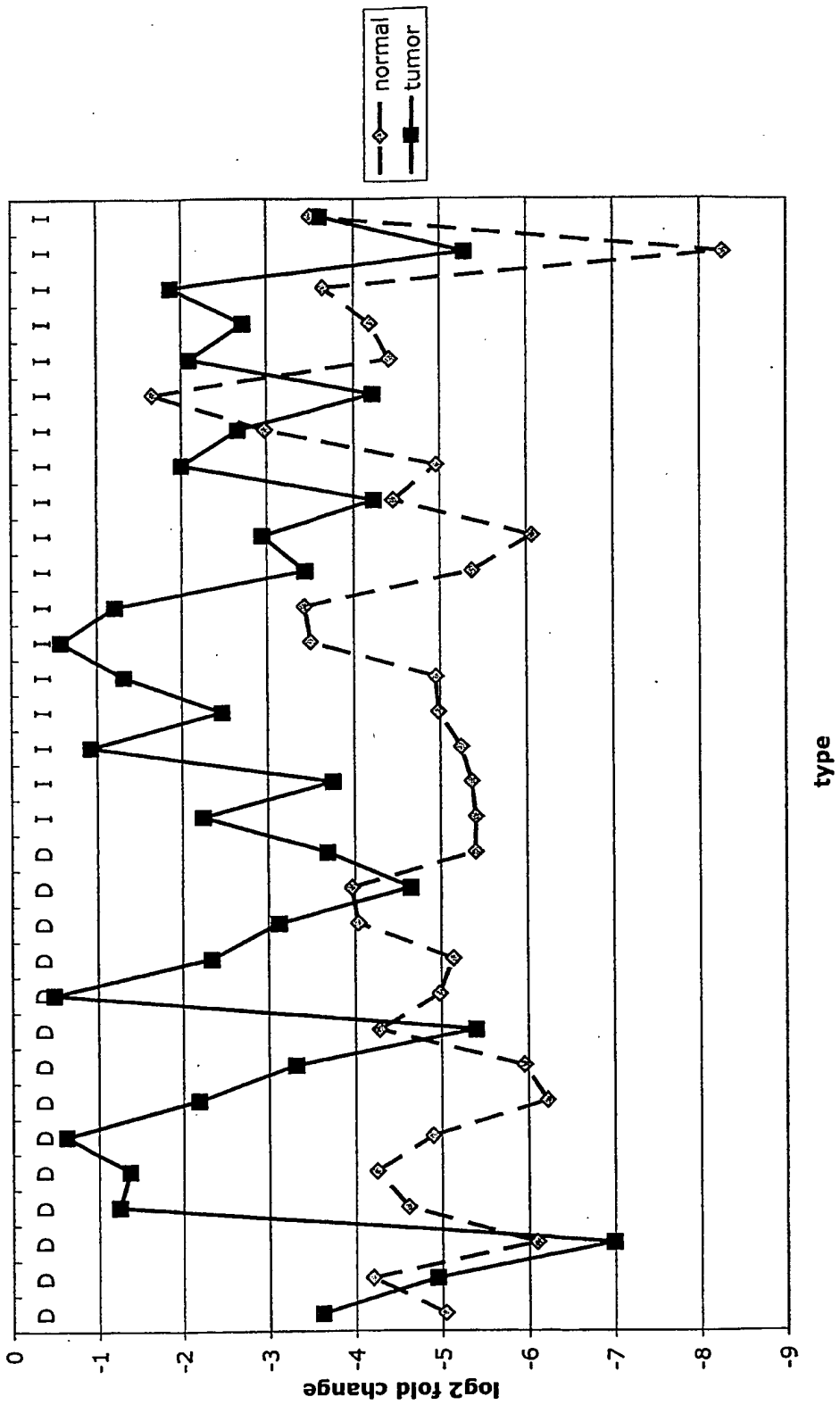

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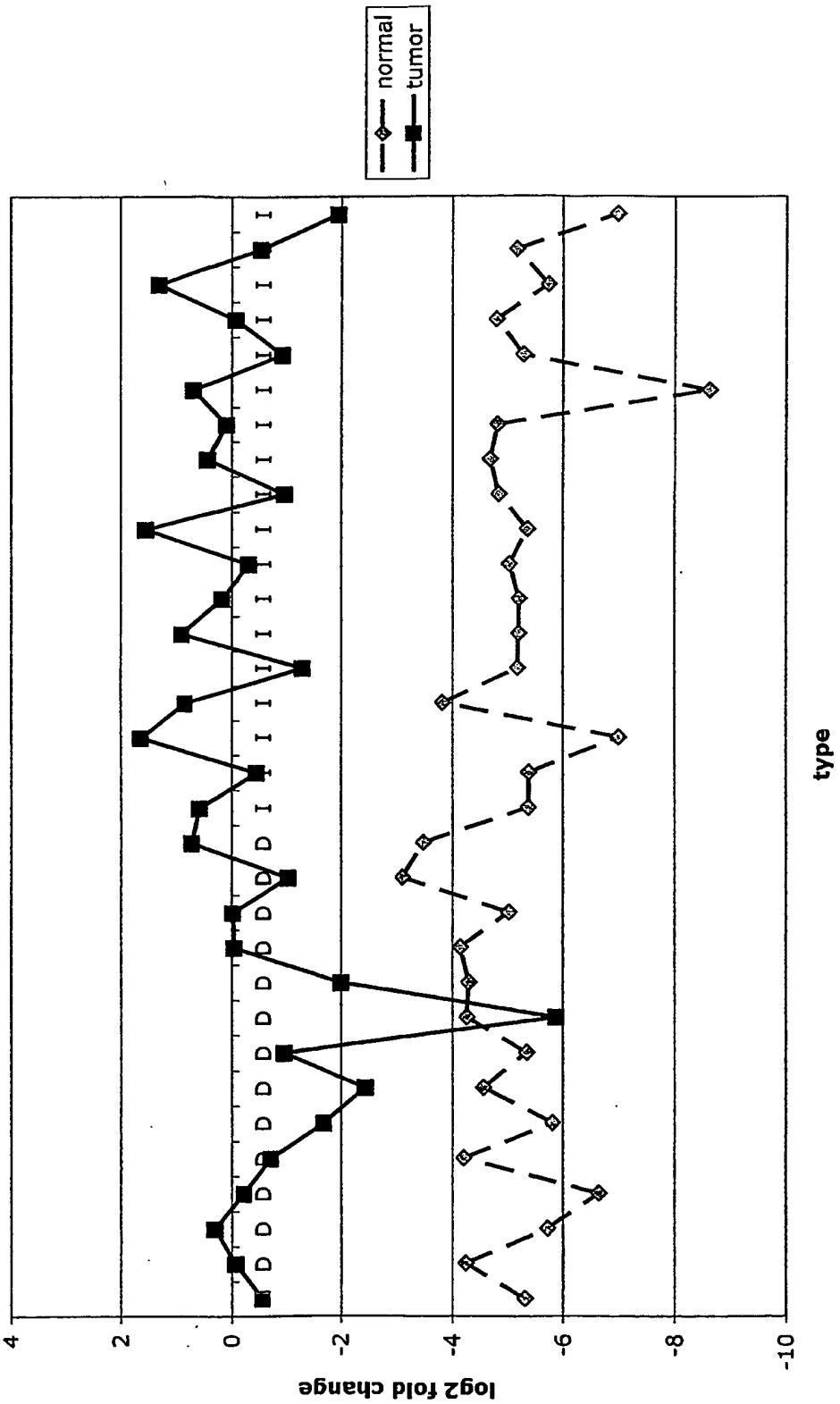

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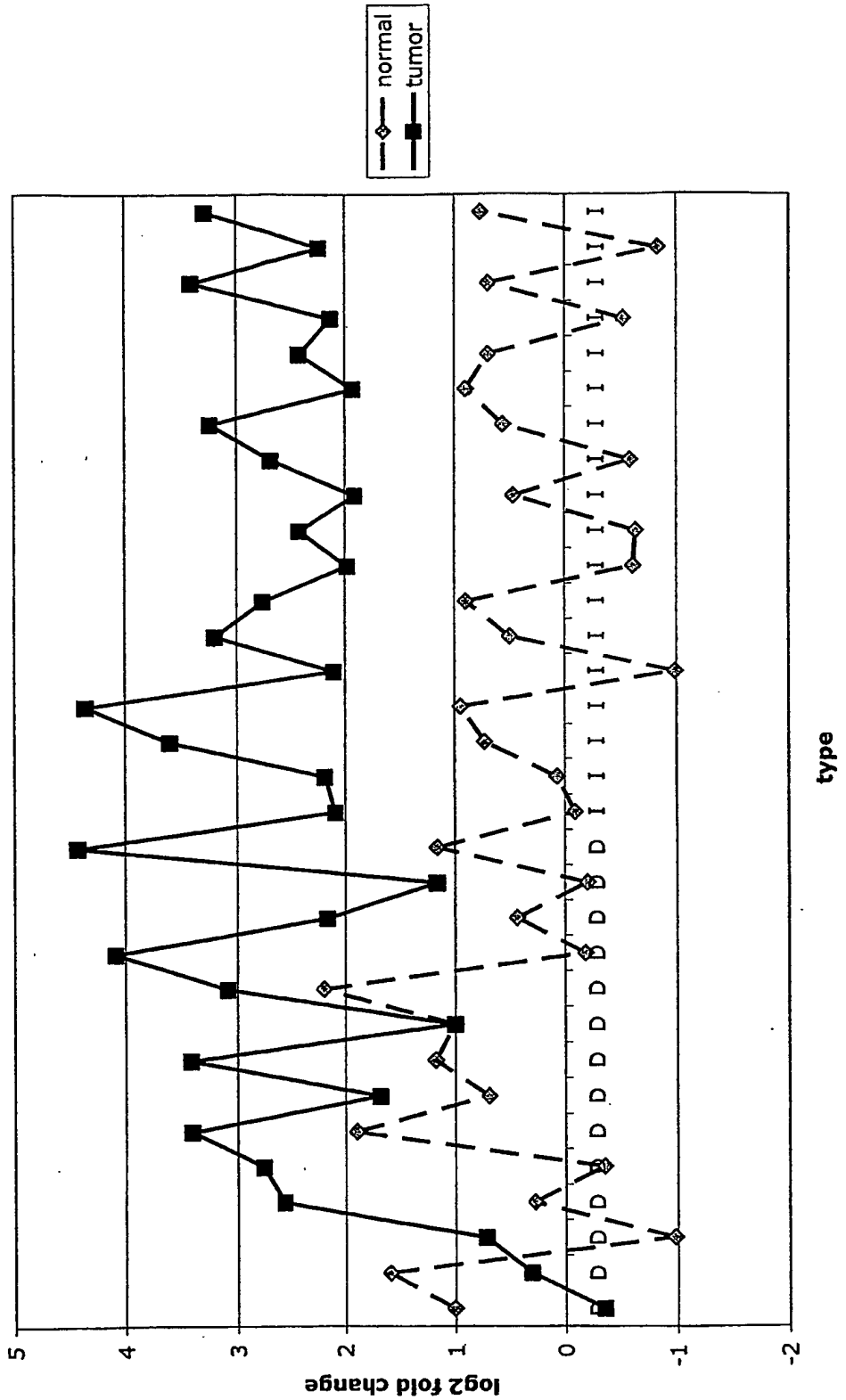

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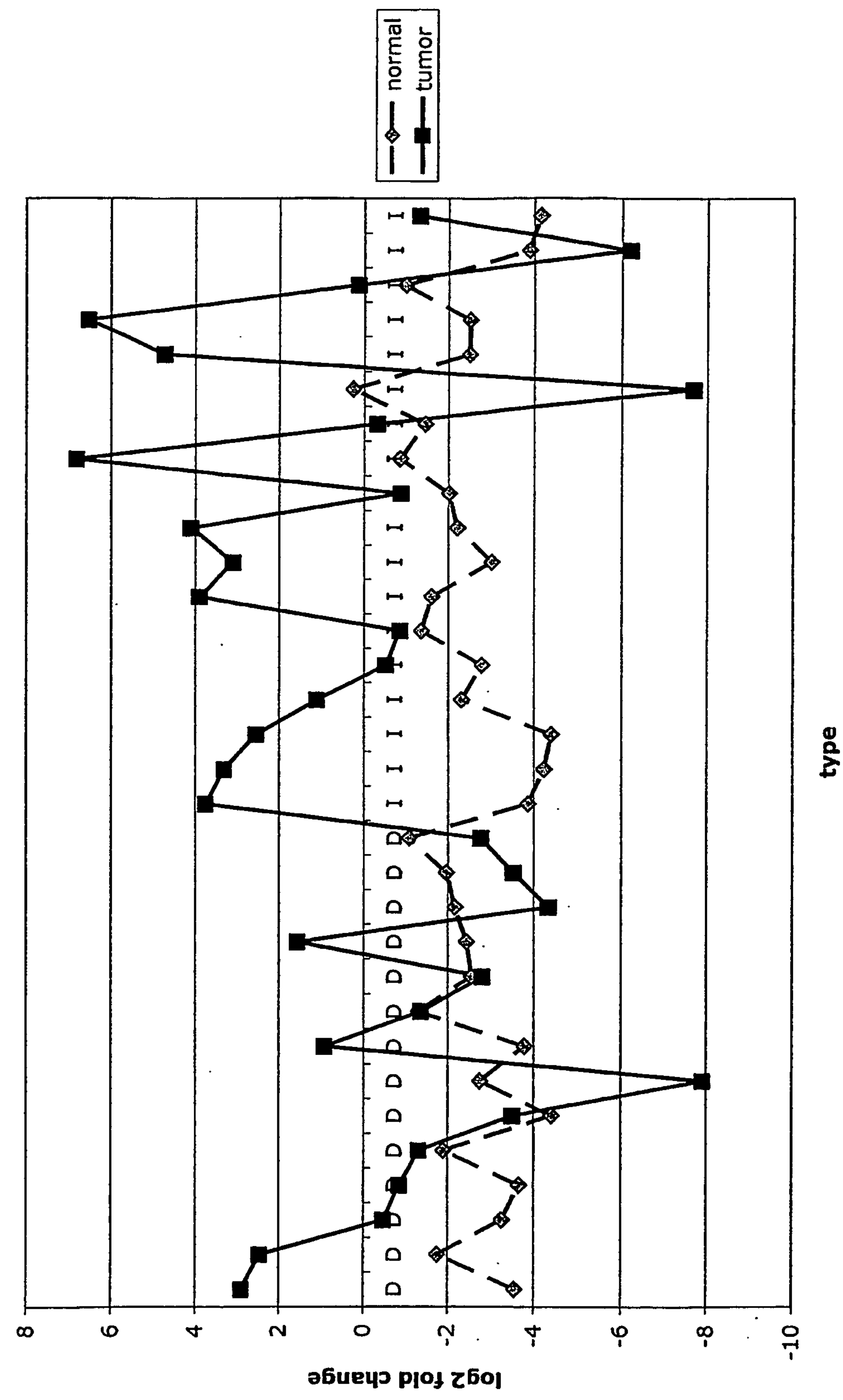

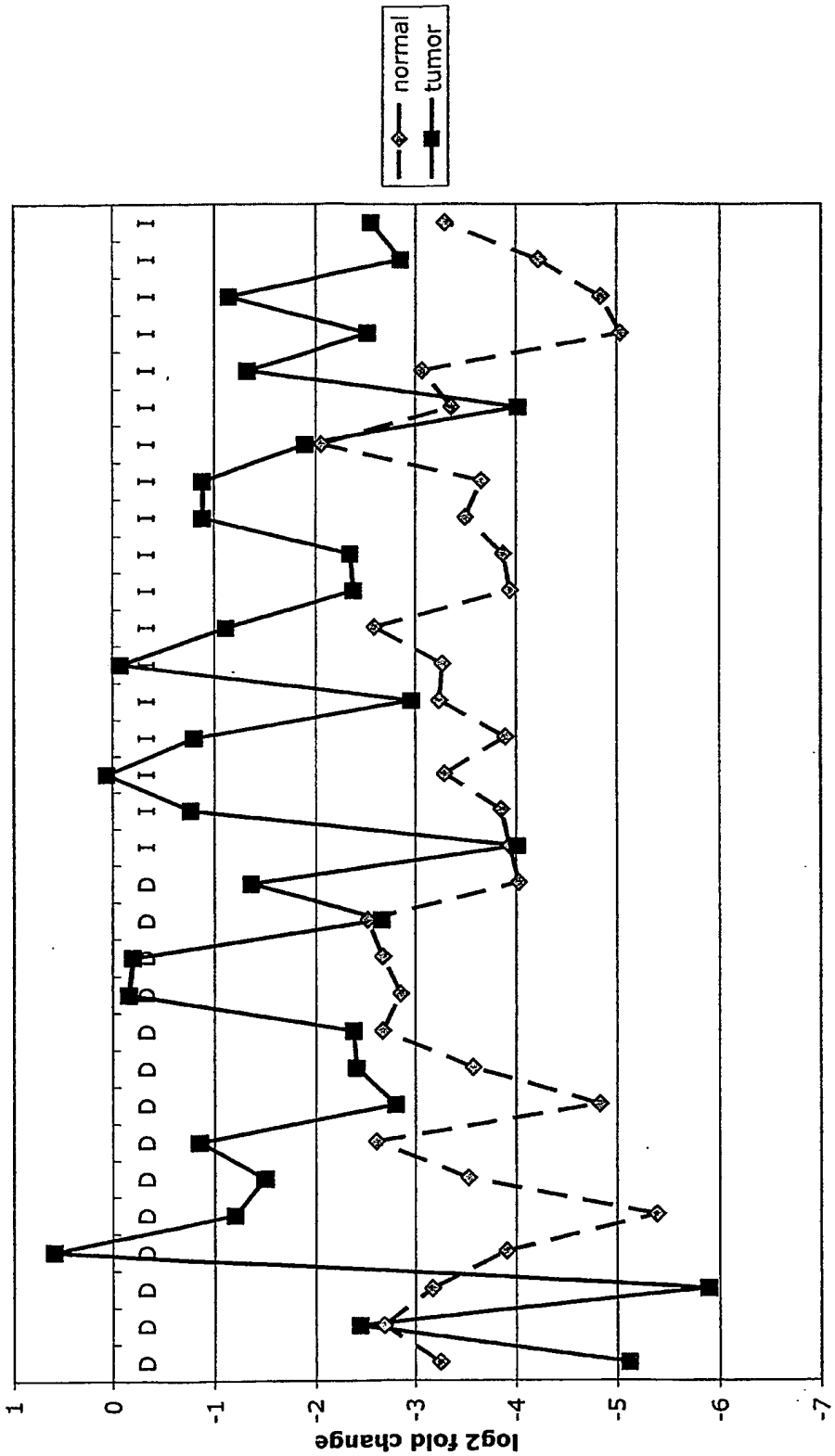

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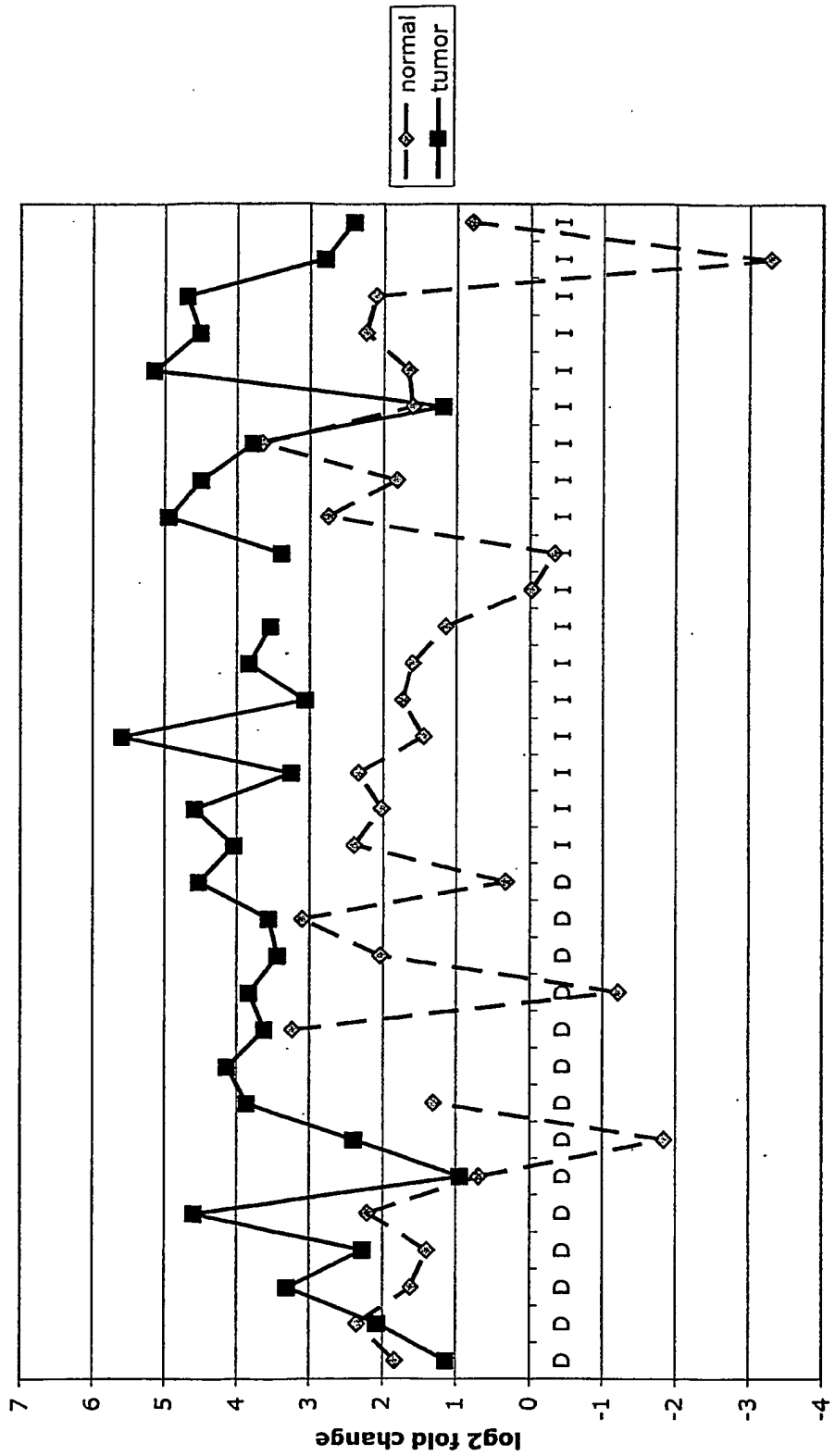

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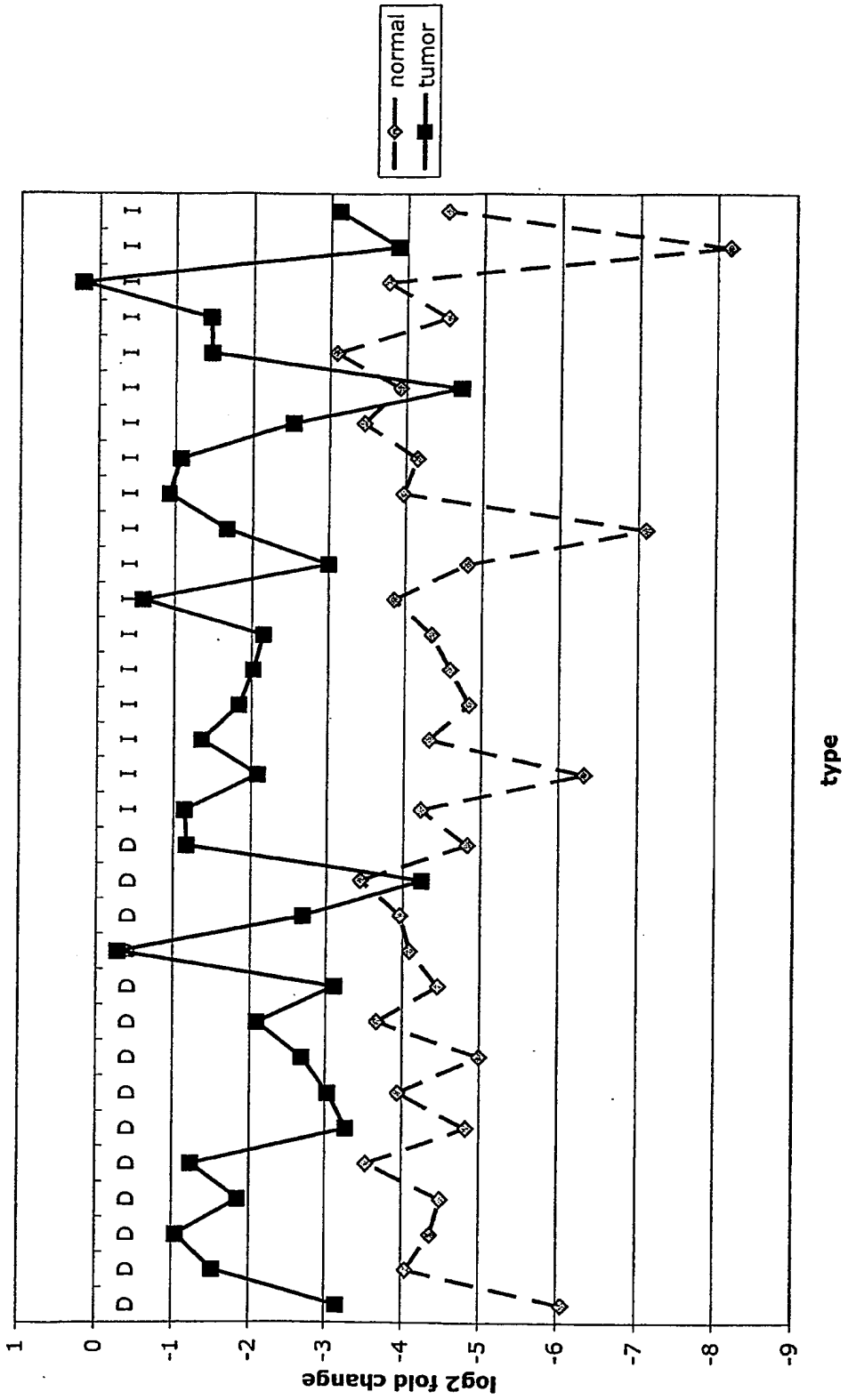
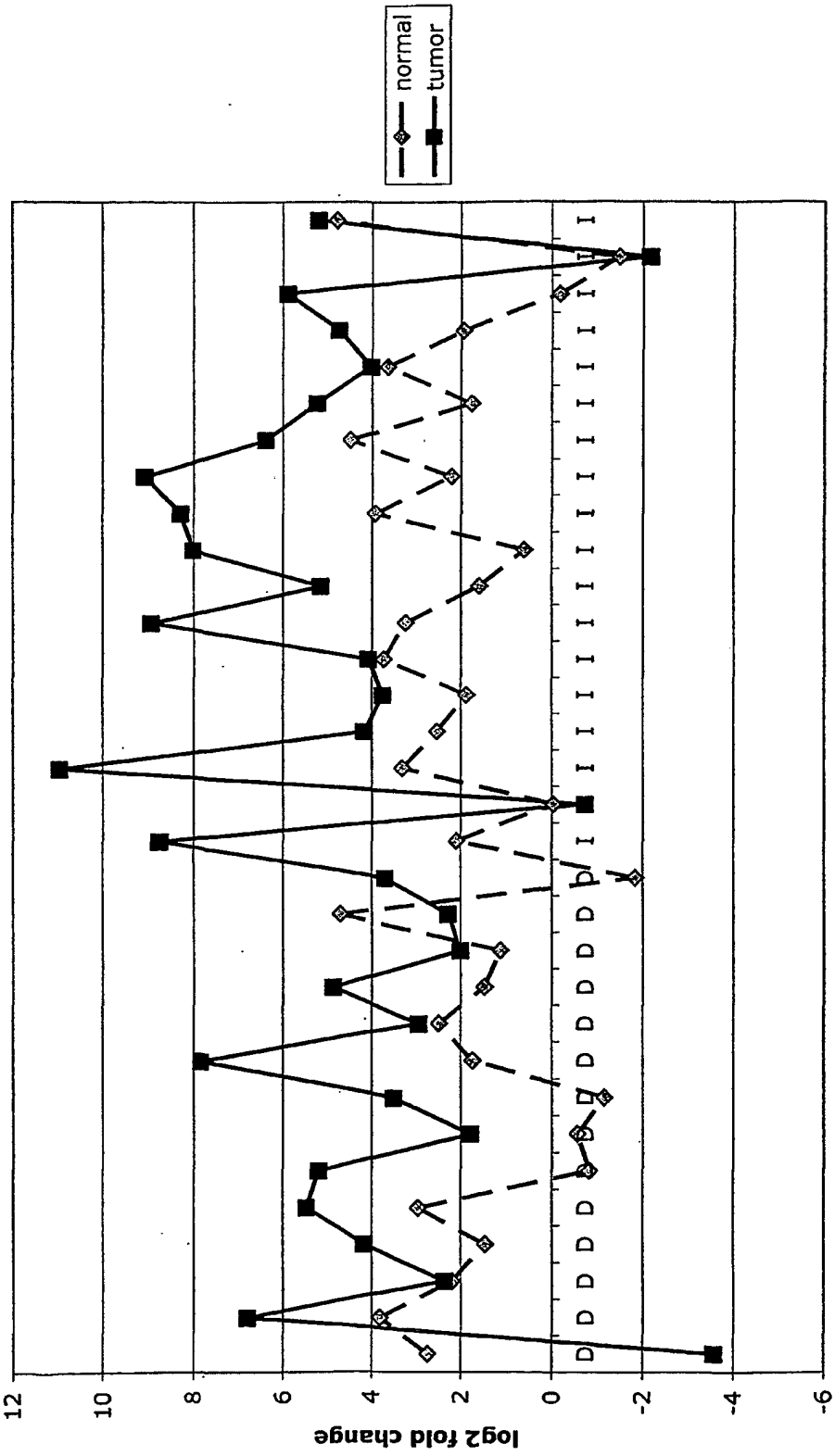
Fig. 11I LOXL2





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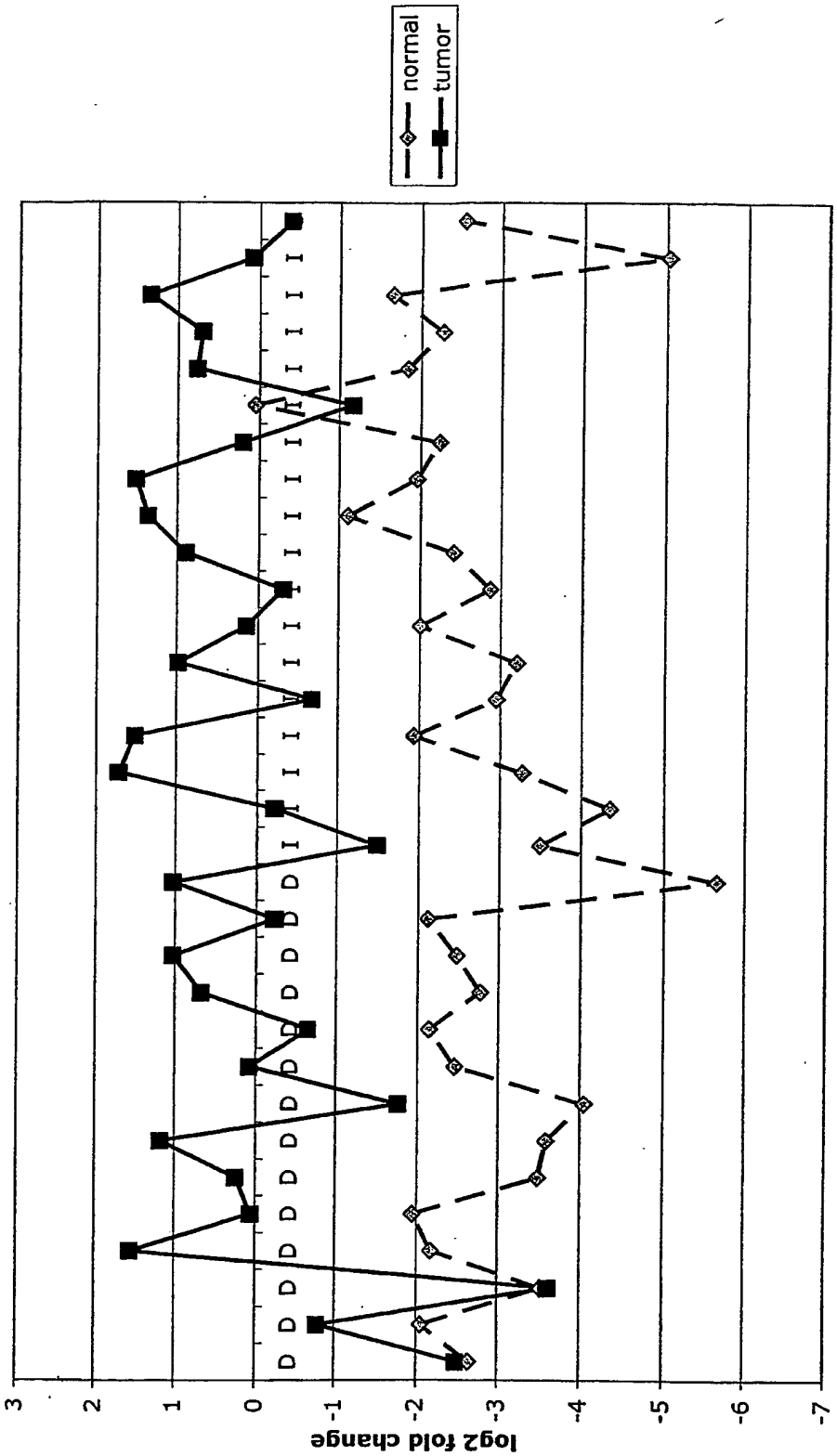

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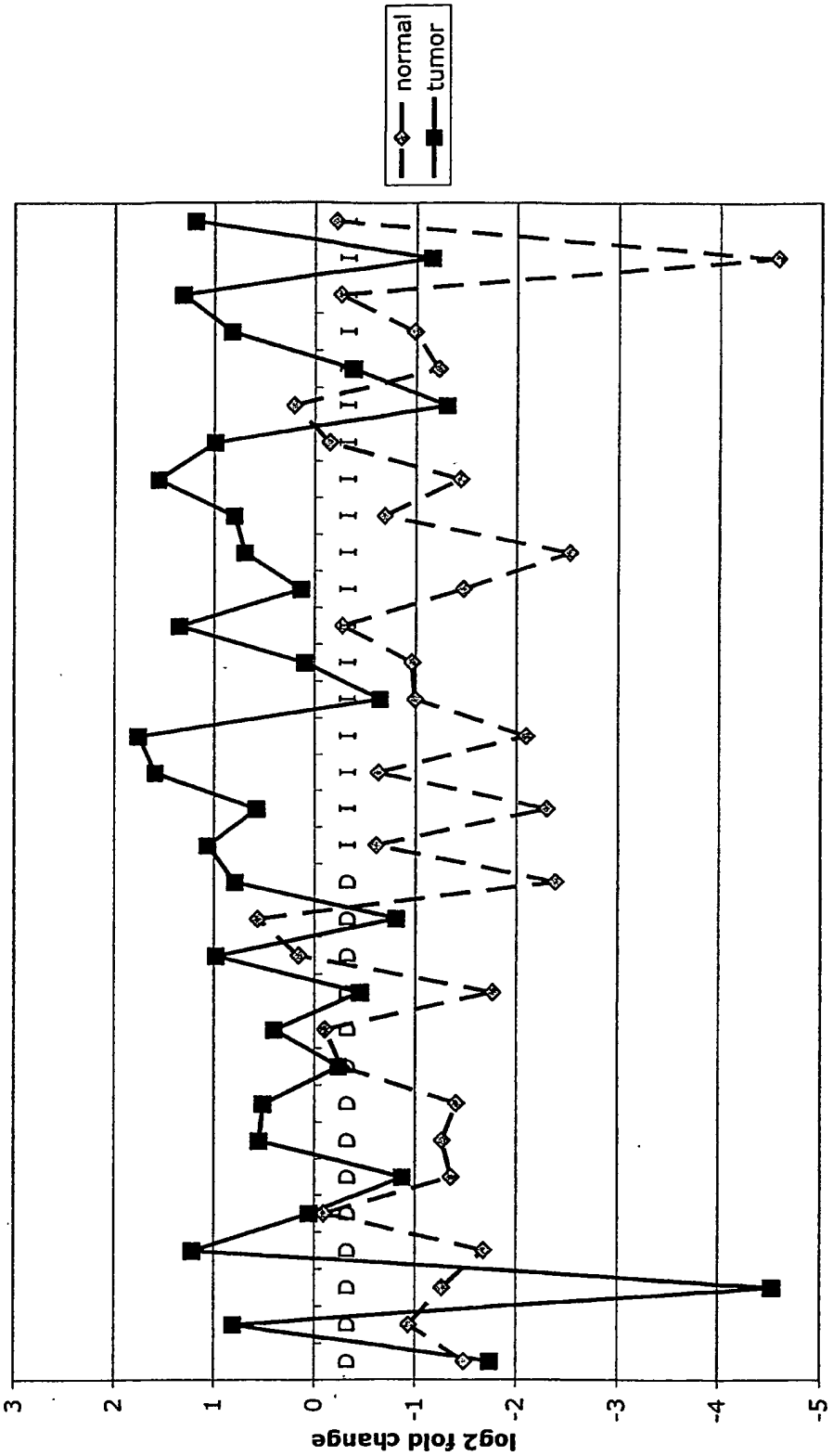

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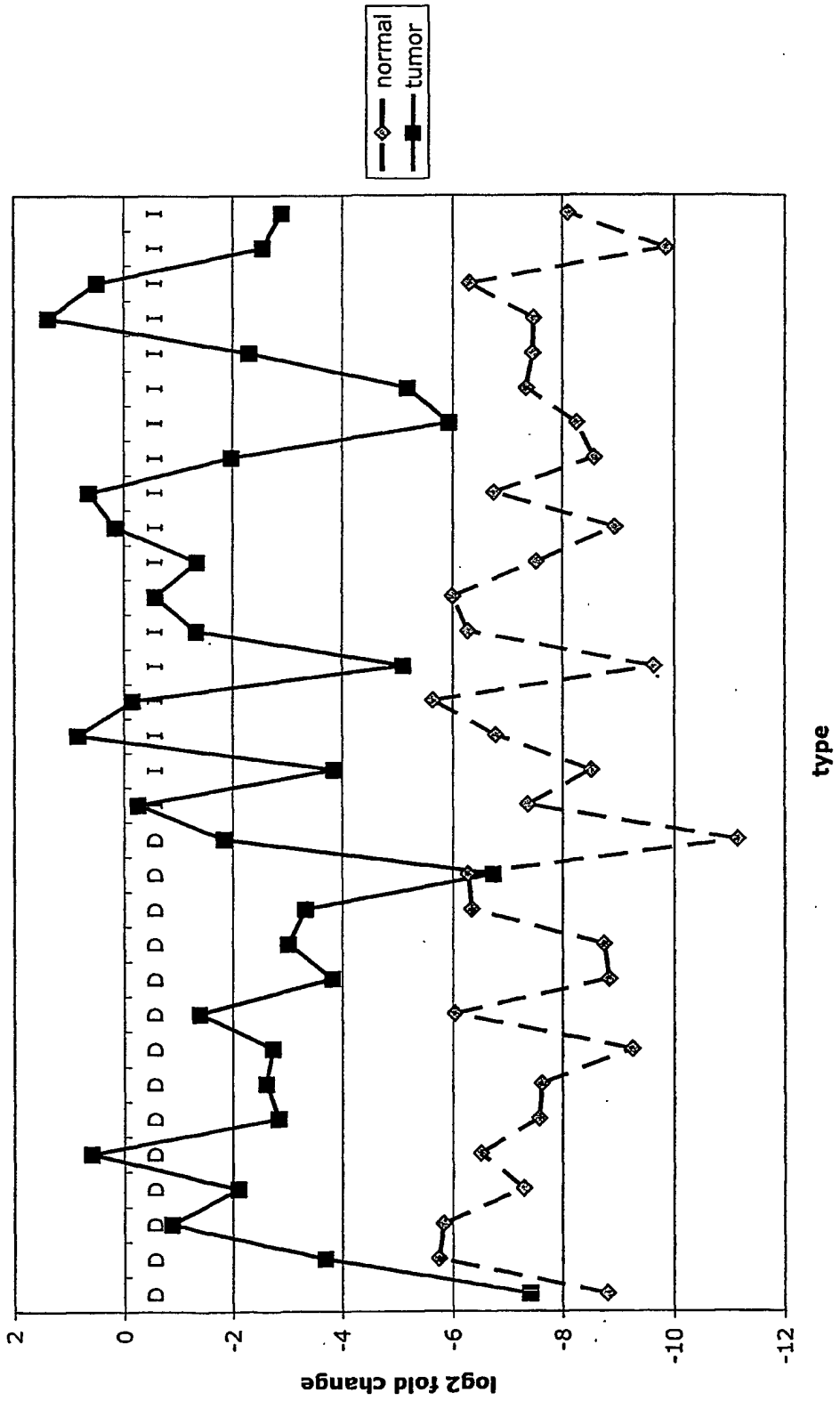

Fig. 11p SPP1

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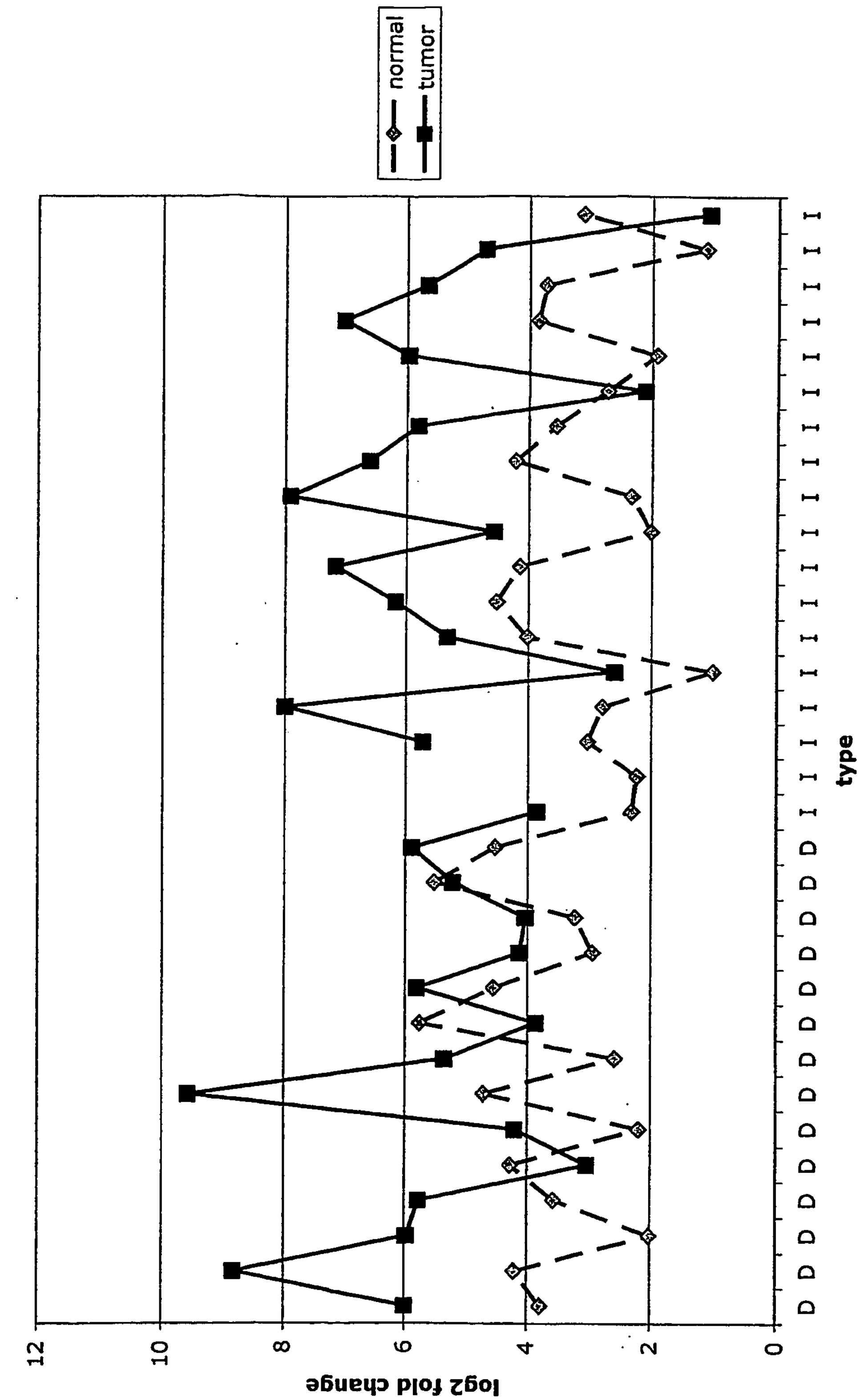

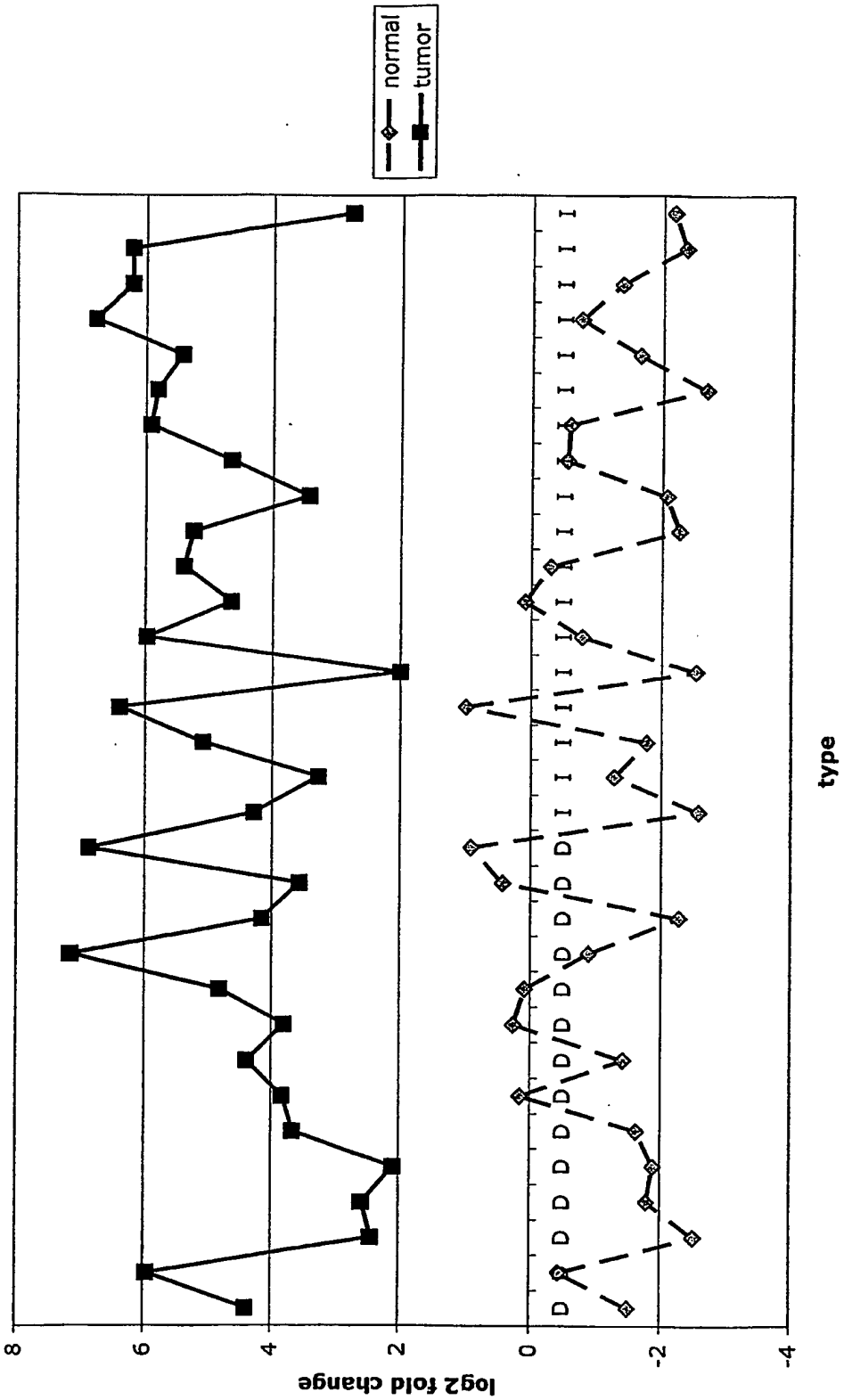

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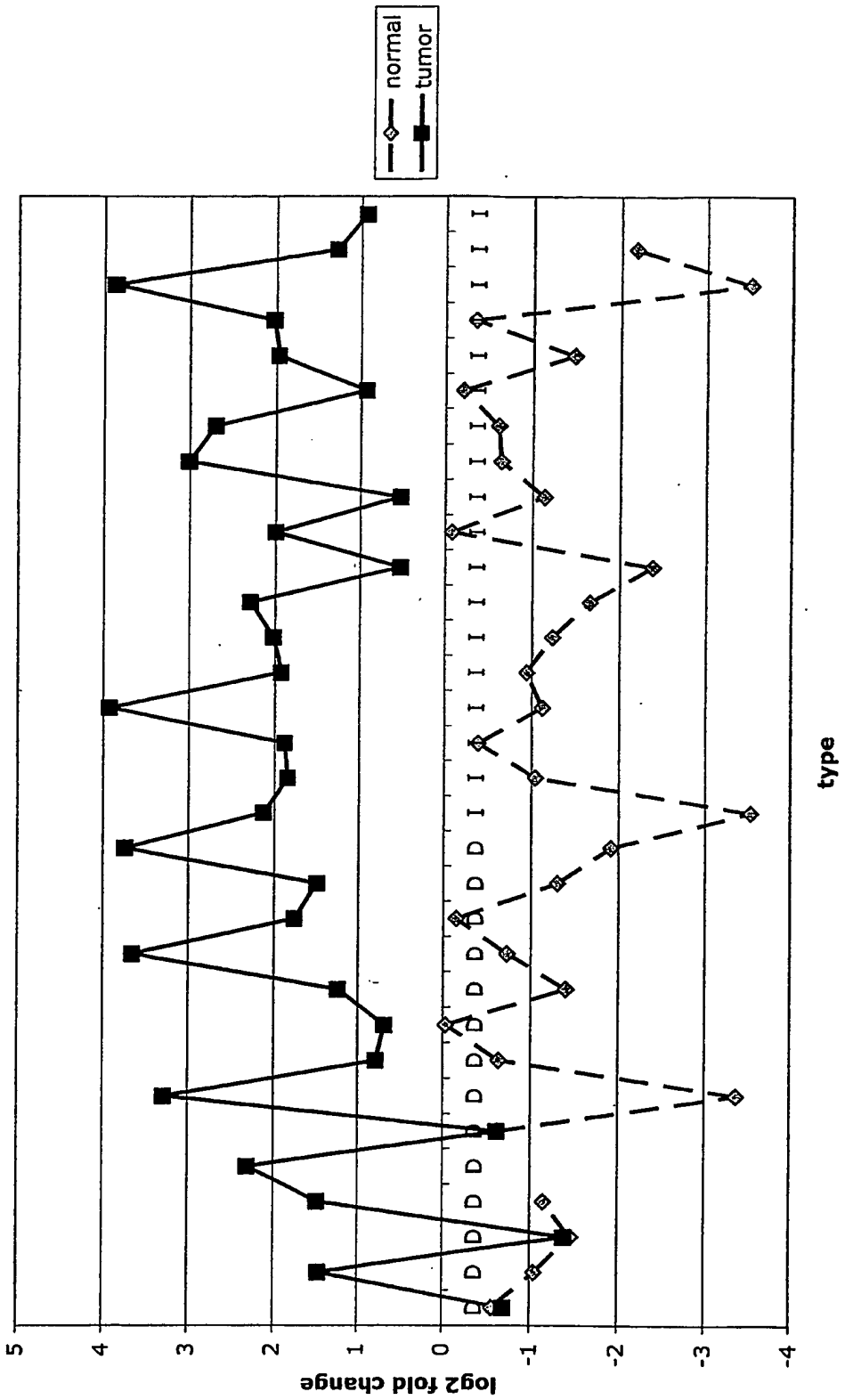

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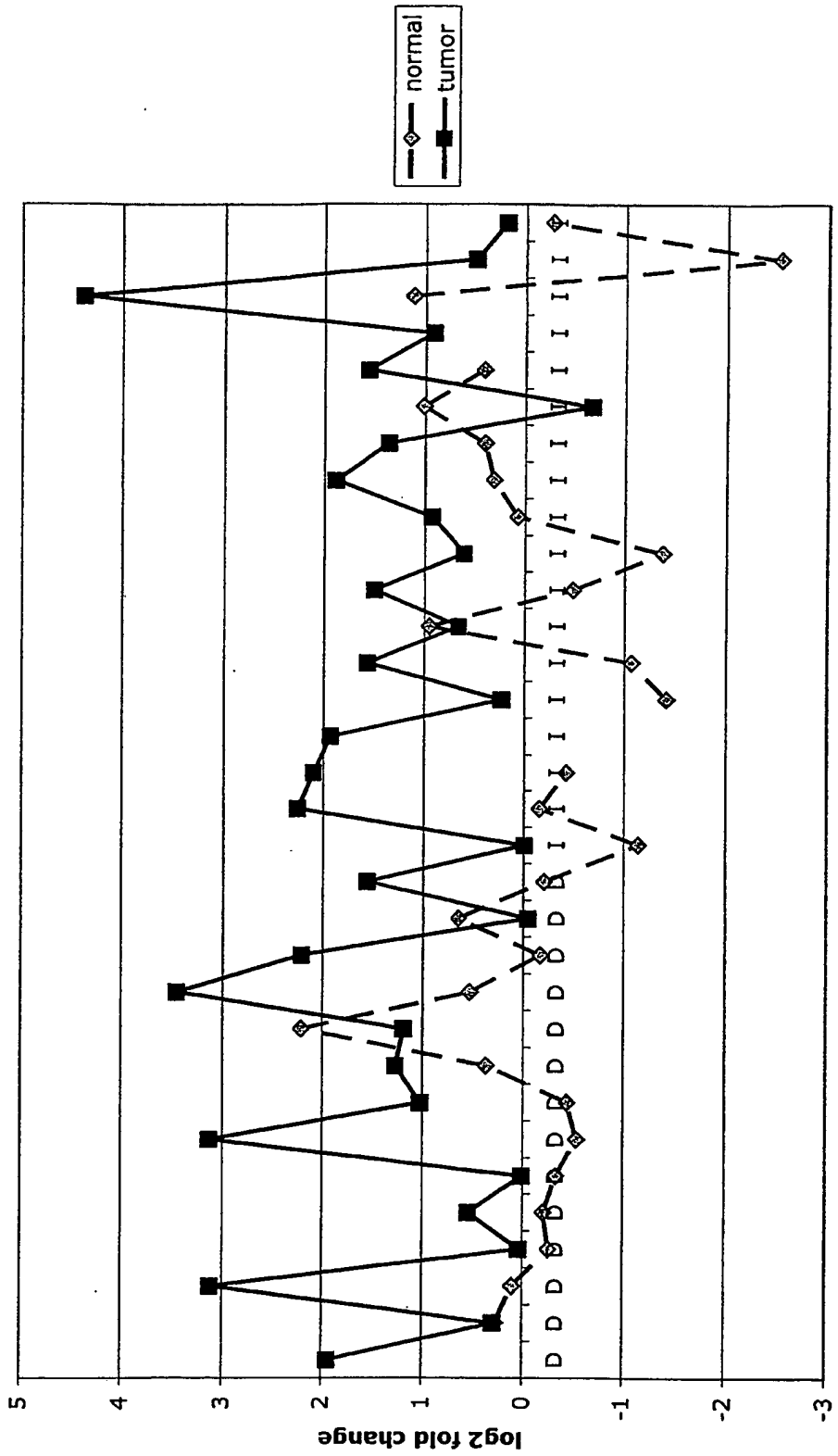

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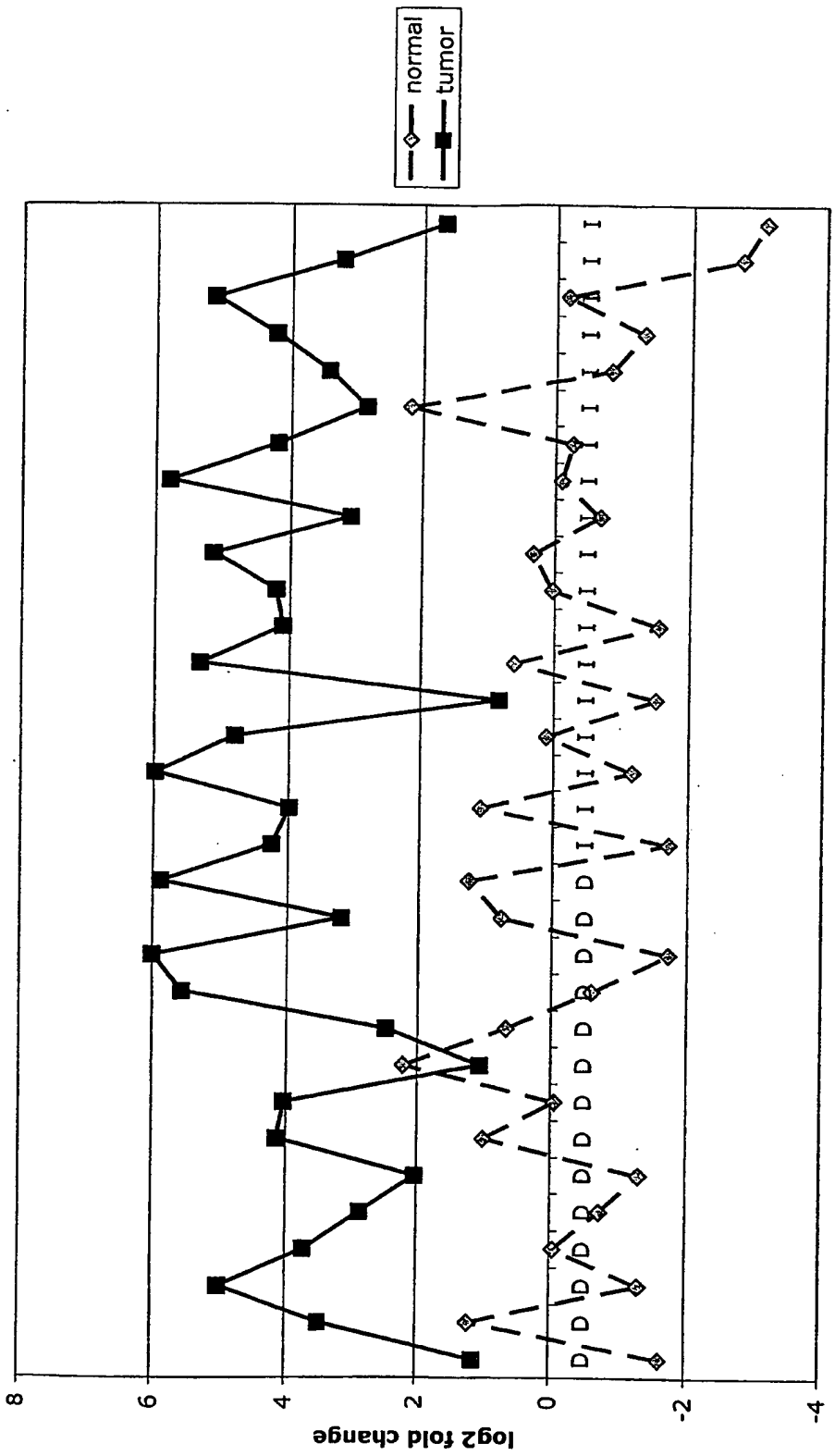

Fig. 11u THBS2

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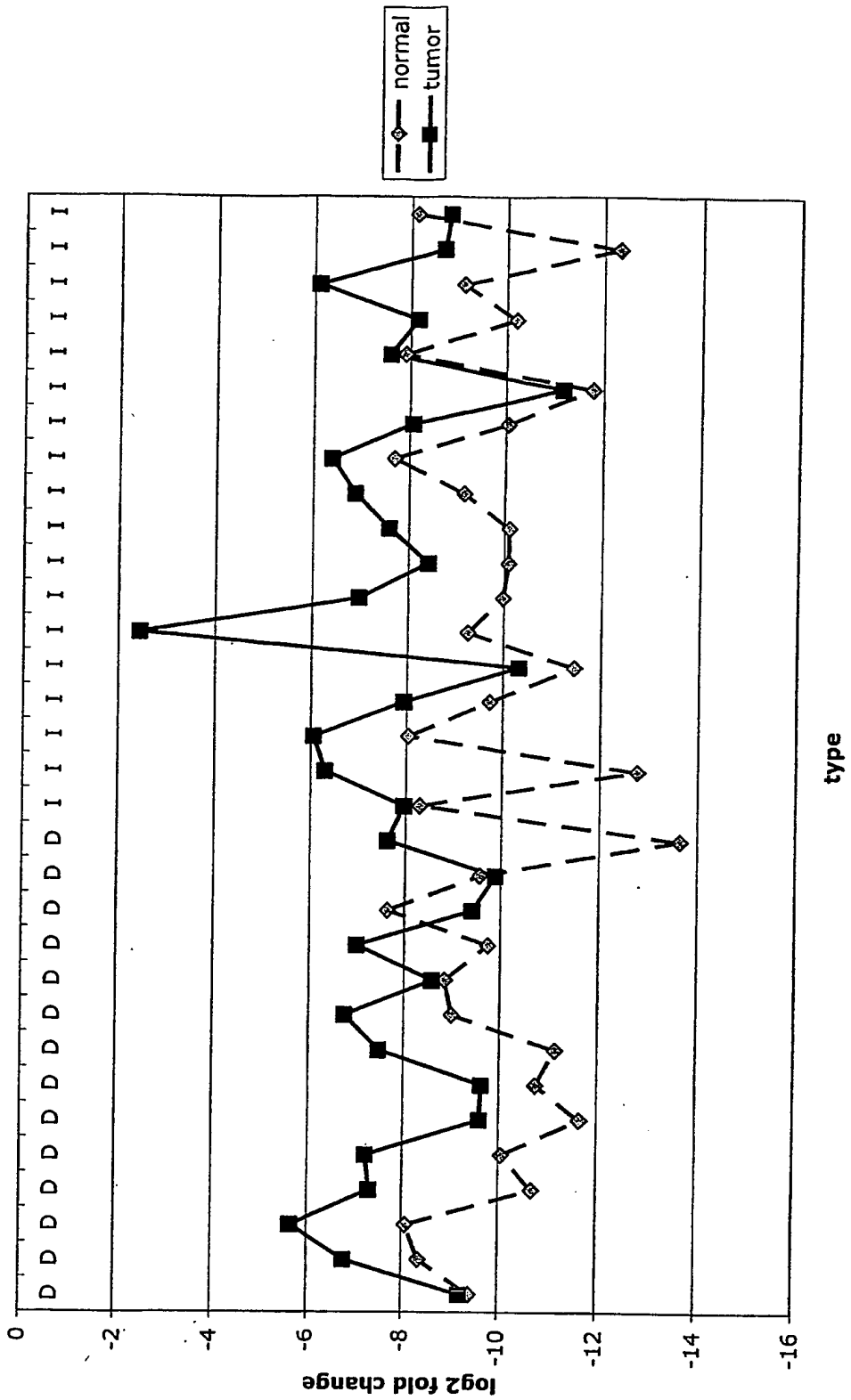

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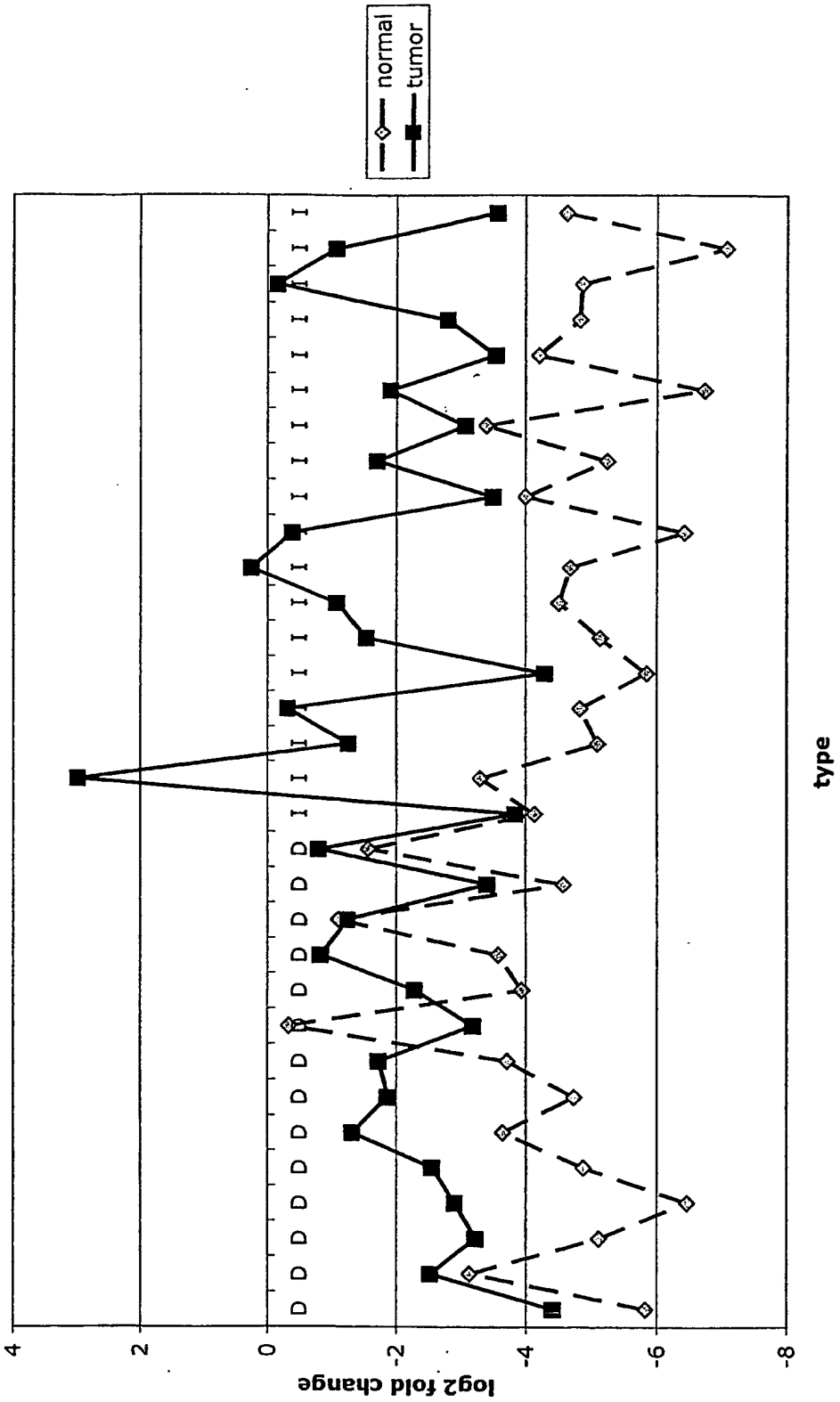

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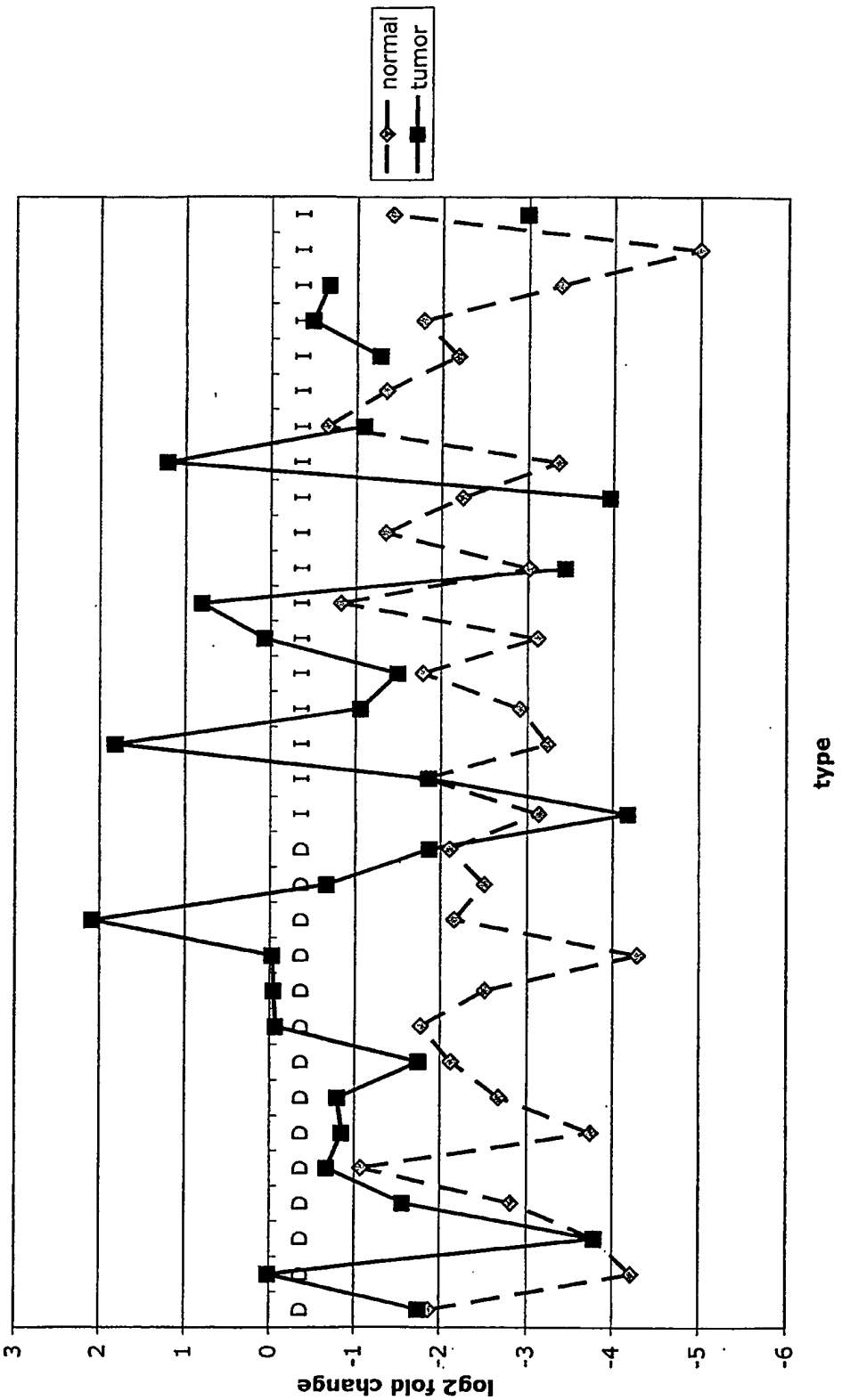


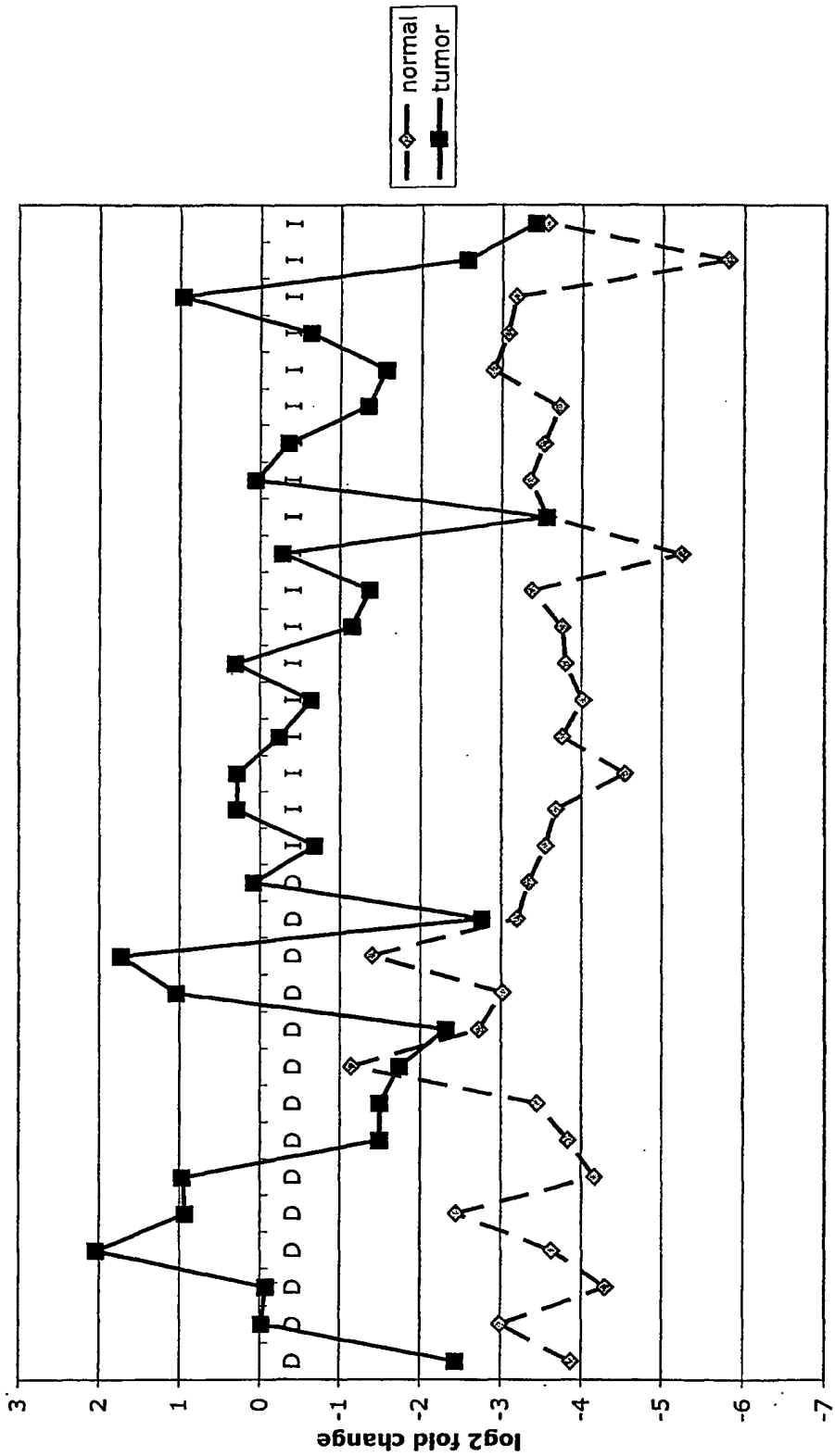


Fig. 11y SERPINH1

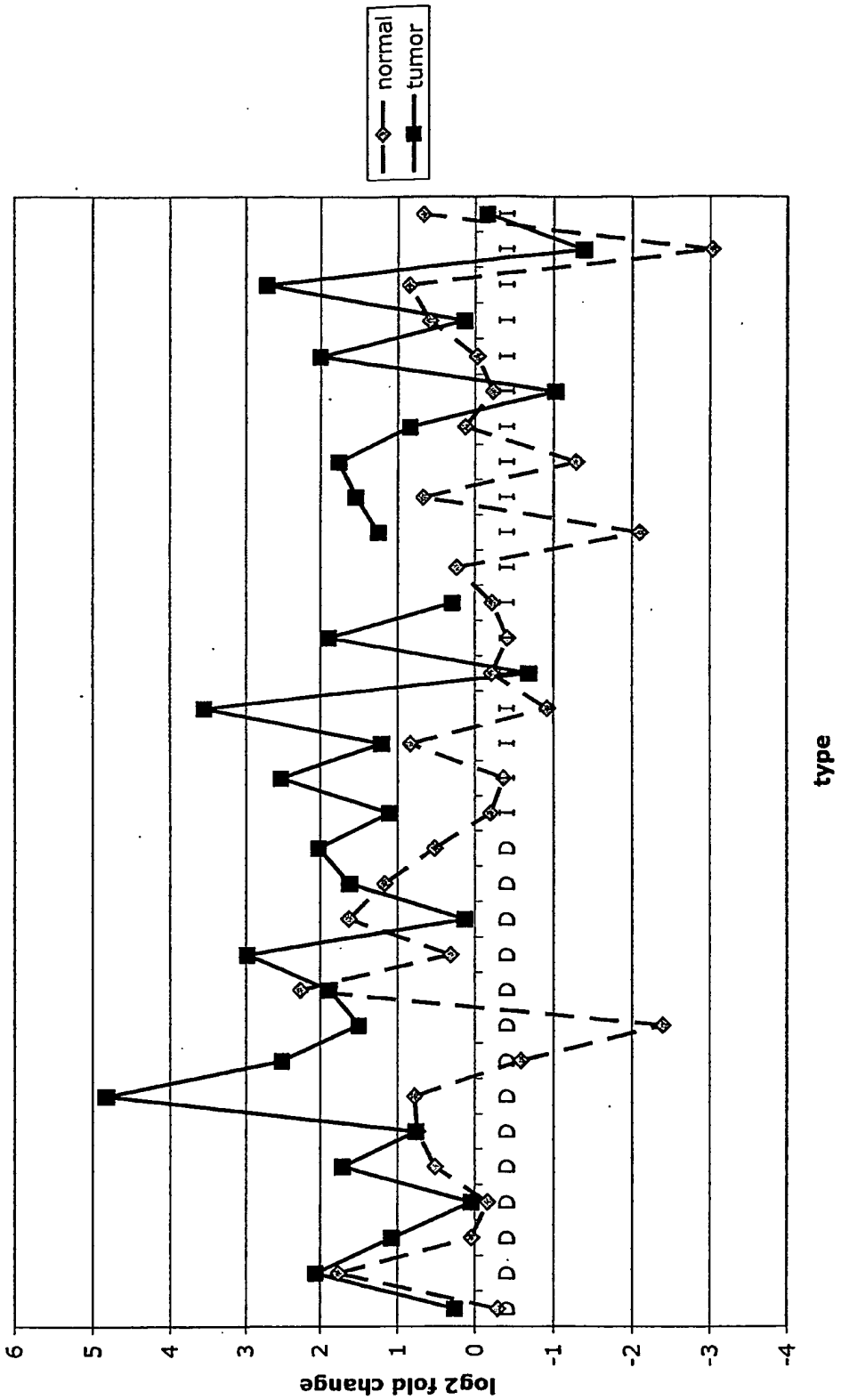


Fig. 11z MMP2

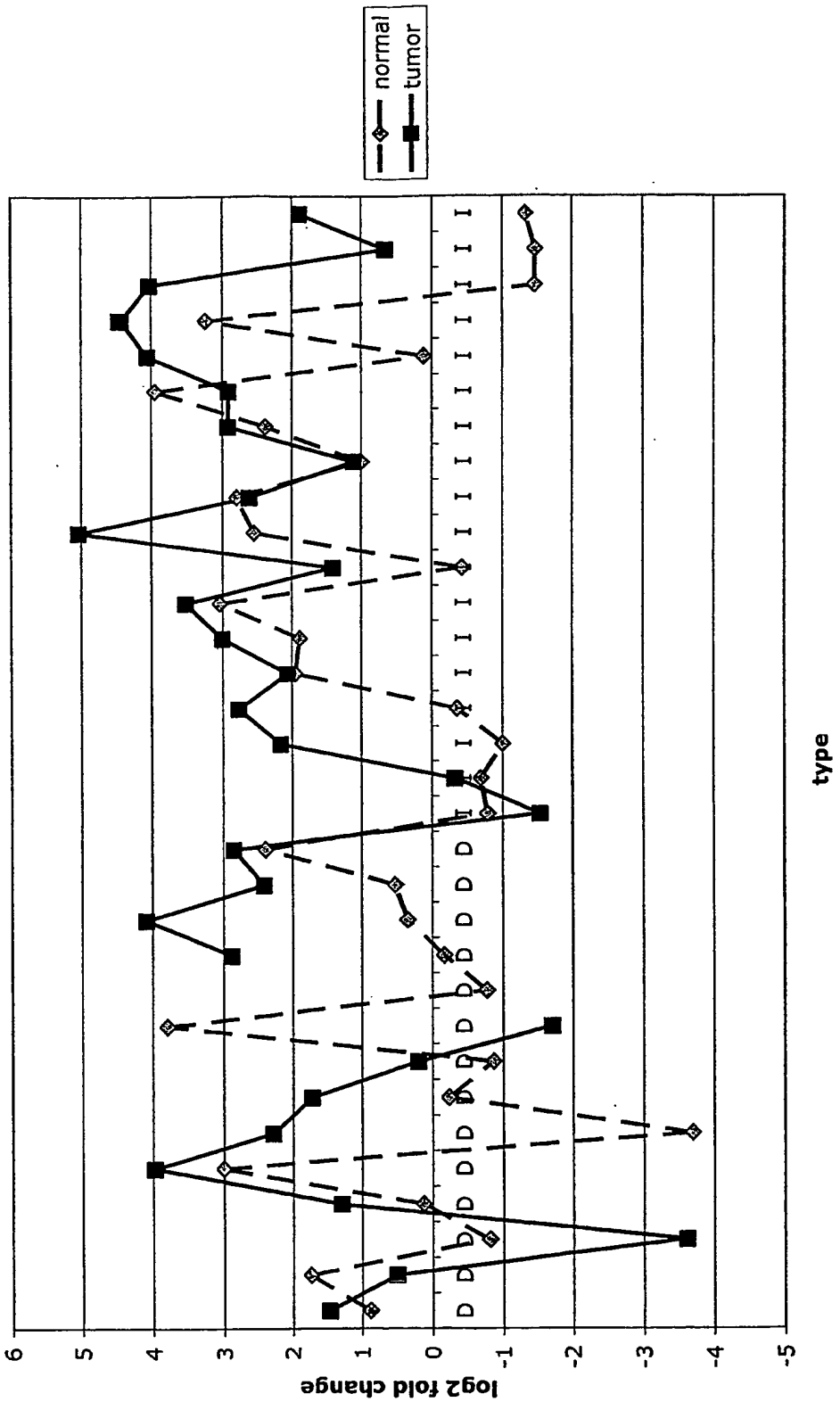
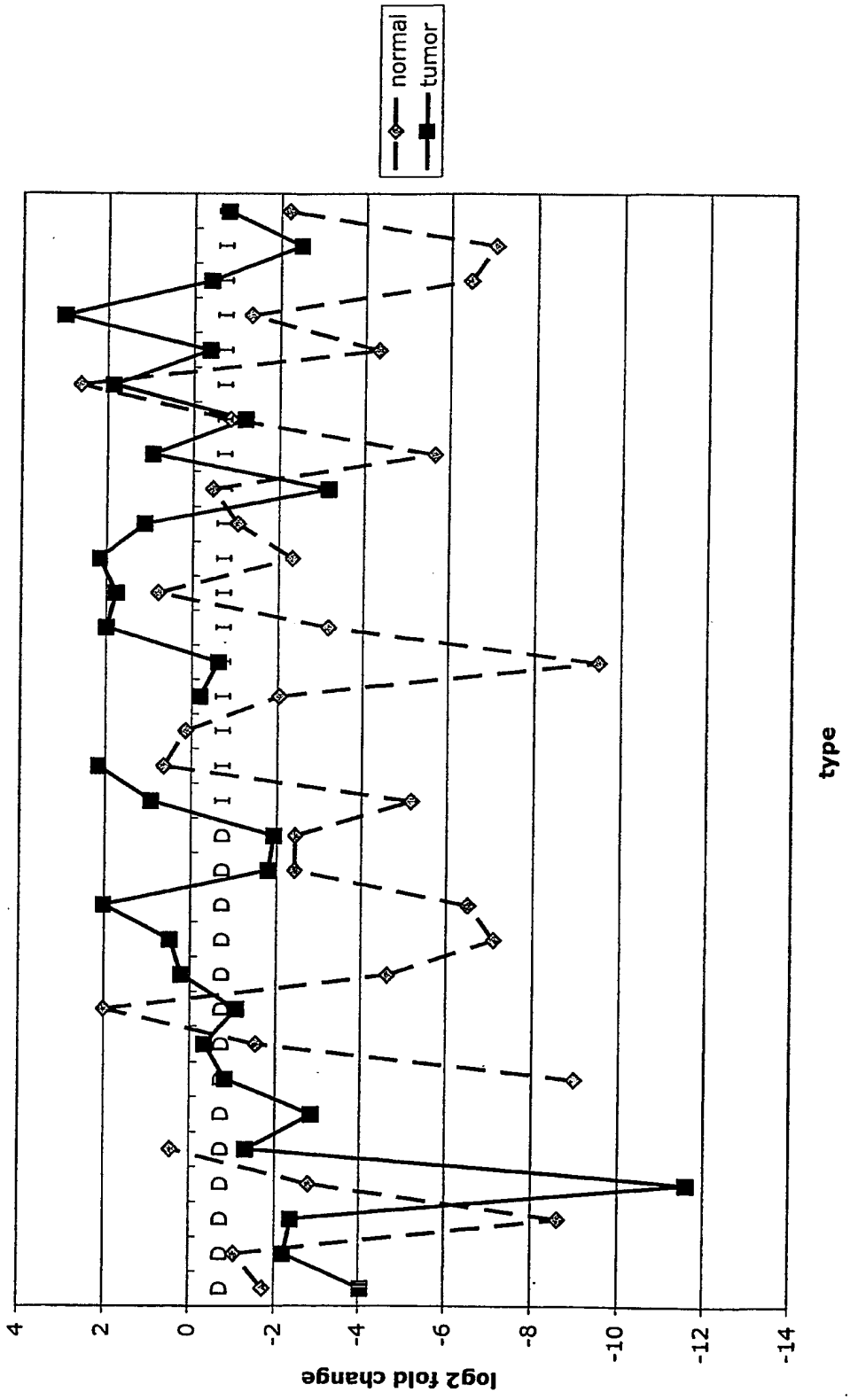


Fig. 11aa PCSK5

Fig. 11ab SERPINB5



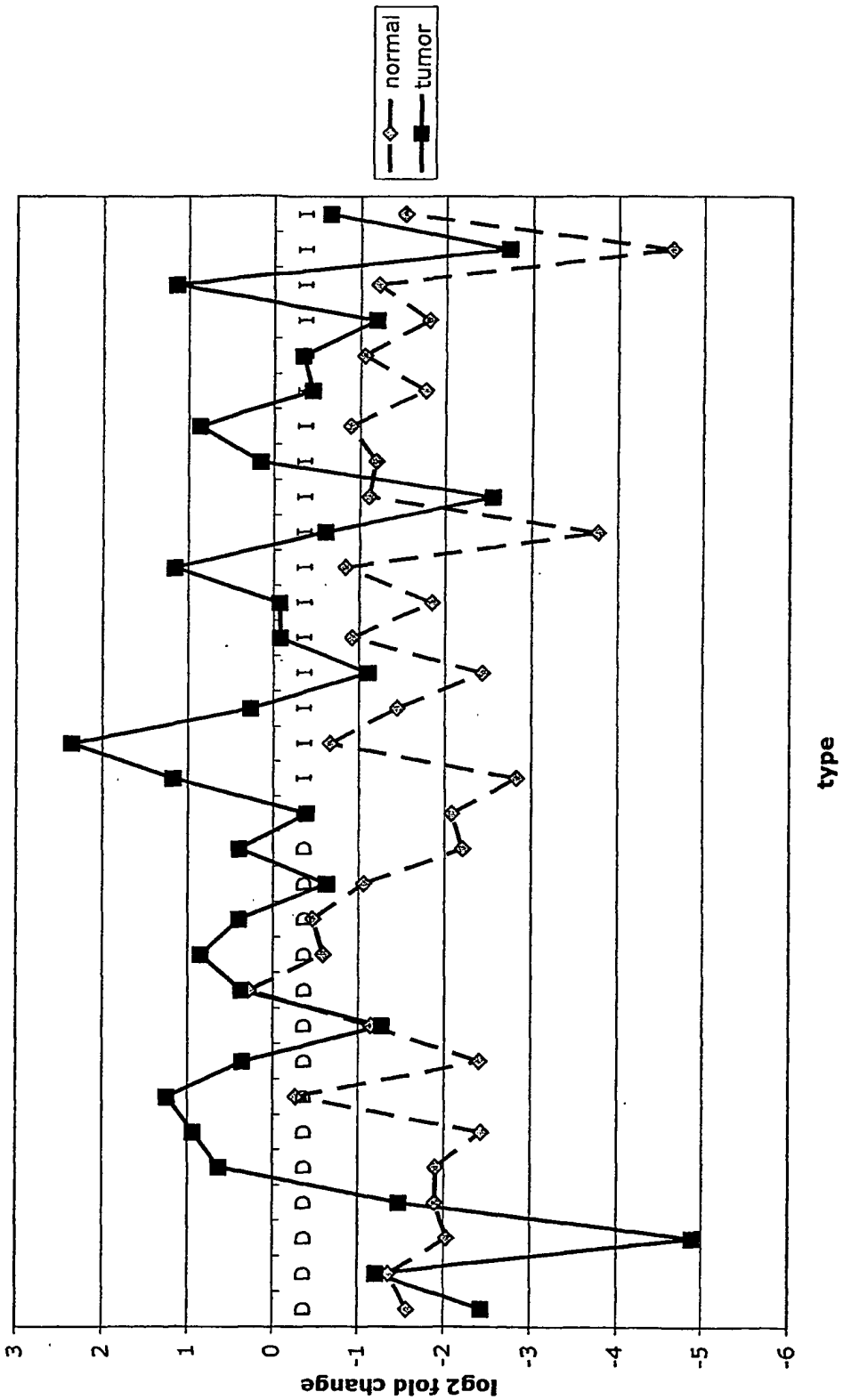


Fig. 11ac TGFB1

Fig. 11ad CEACAM5

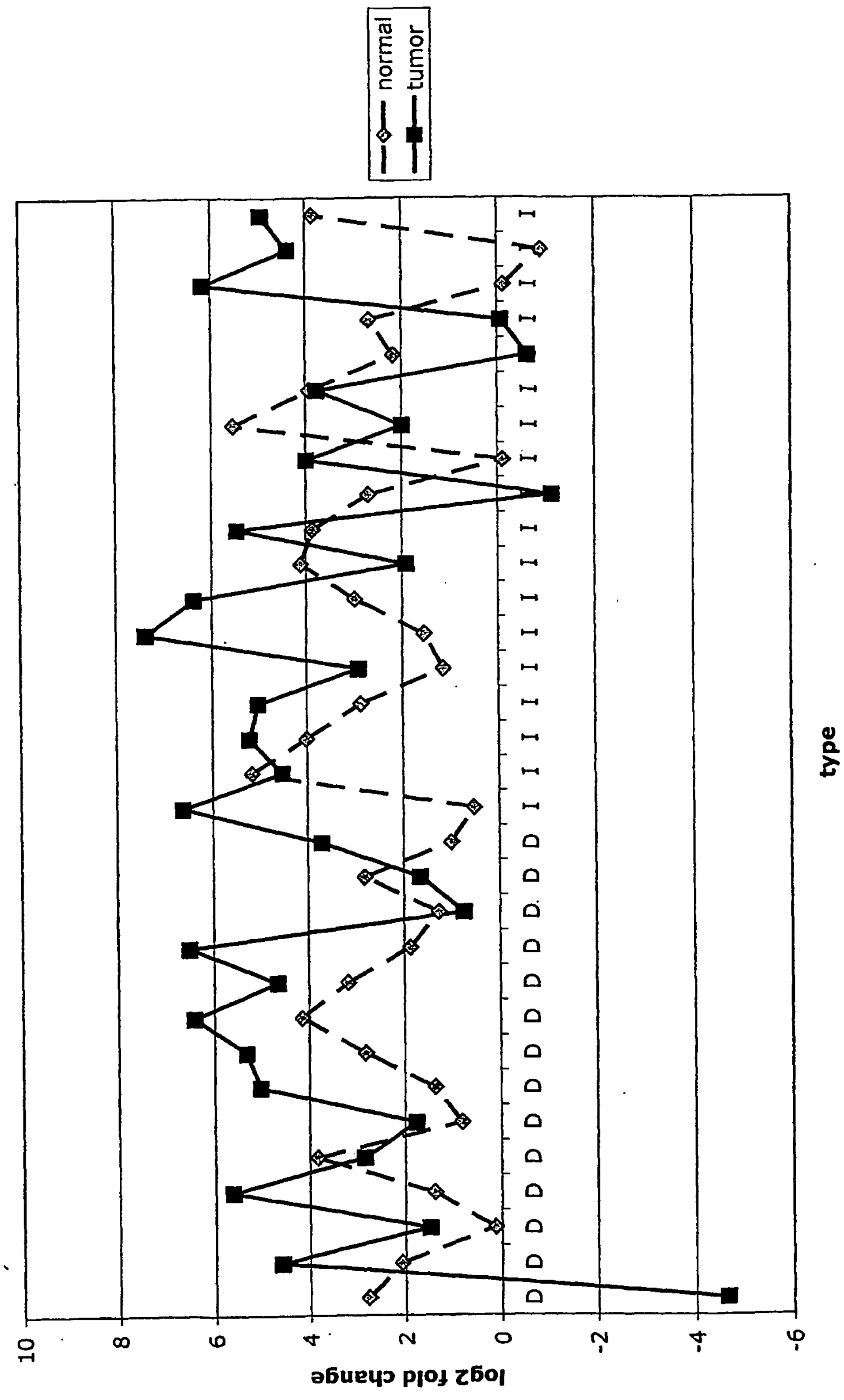
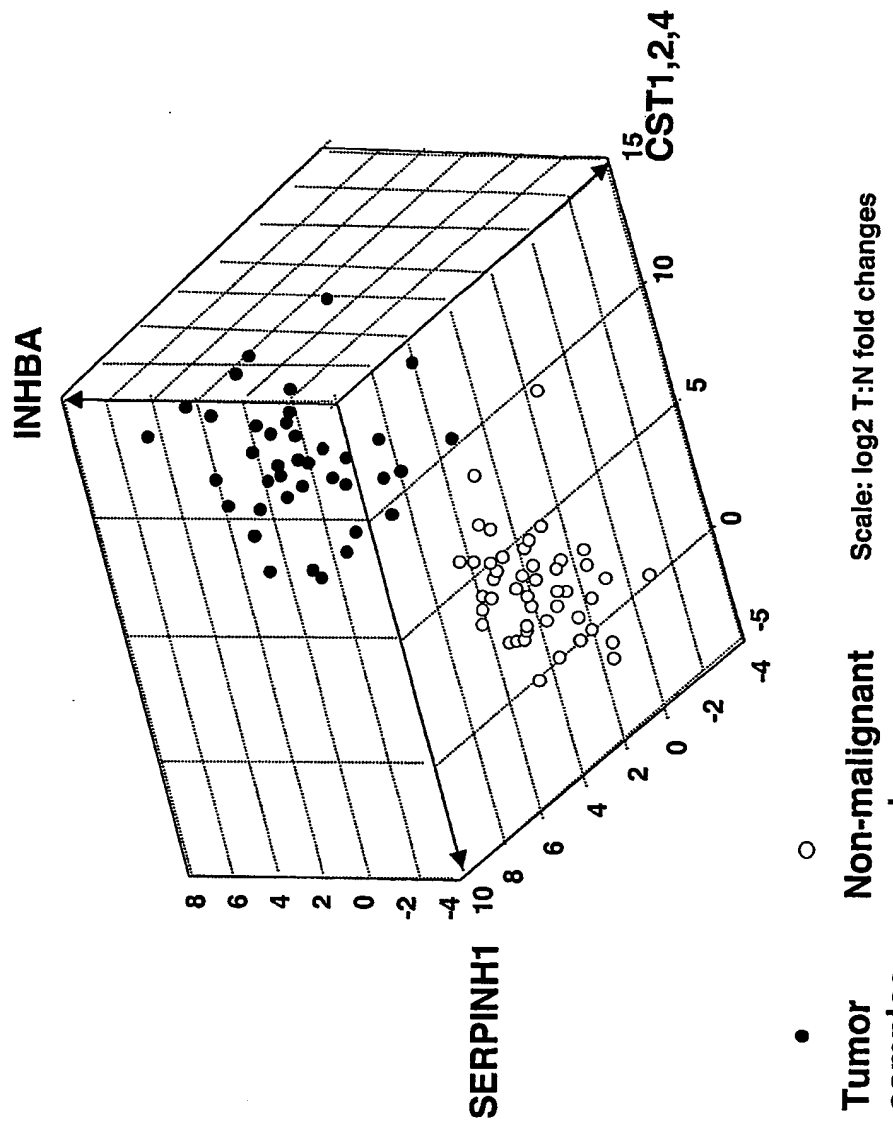


Fig. 12 The separation of gastric tumor samples from non-malignant samples using three markers



Number of markers in test	Total possible tests	Number of tests with sensitivity		Proportion of tests with sensitivity			
		>=90%	>=95%	>=99%	>=90%	>=95%	>=99%
1	29	2	1	0	6.9%	3.4%	0%
2	406	33	27	1	8.1%	6.7%	0.2%
3	3654	796	457	50	21.8%	12.5%	1.4%

Fig. 13. The effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue.

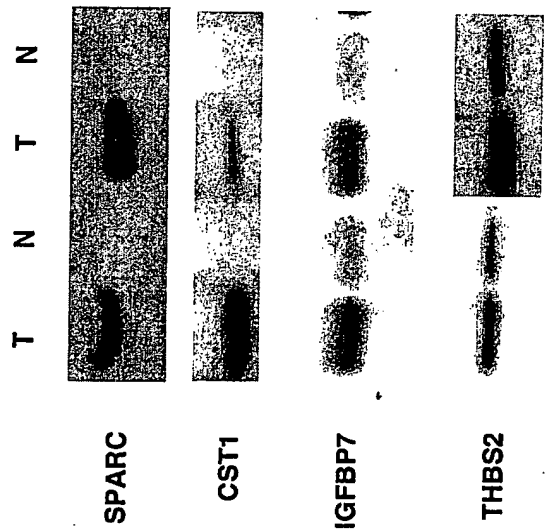


Fig. 14. Western analysis of markers in tumor and non-malignant tissue

Fig. 15. Western analysis of SPARC in gastric tumor material and serum.

Fig. 16. Immunodetection of cystatin SN in the supernatant of the gastric cancer cell line, AGS.

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Guilford, Parry J.
Holyoake, Andrew J.

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